

Proceedings of

INTERNATIONAL CME ON TUBERCULOSIS

(Epidemiology, Diagnosis, Treatment & Control)

27th & 28th September 1996

Editors :

Dr. (Mrs.) Pratibha Narang

Dr. D.K. Mendiratta

DEPARTMENT OF MICROBIOLOGY ,
MAHATMA GANDHI INSTITUTE OF MEDICAL SCIENCES,
SEVAGRAM, WARDHA - 442 102 INDIA

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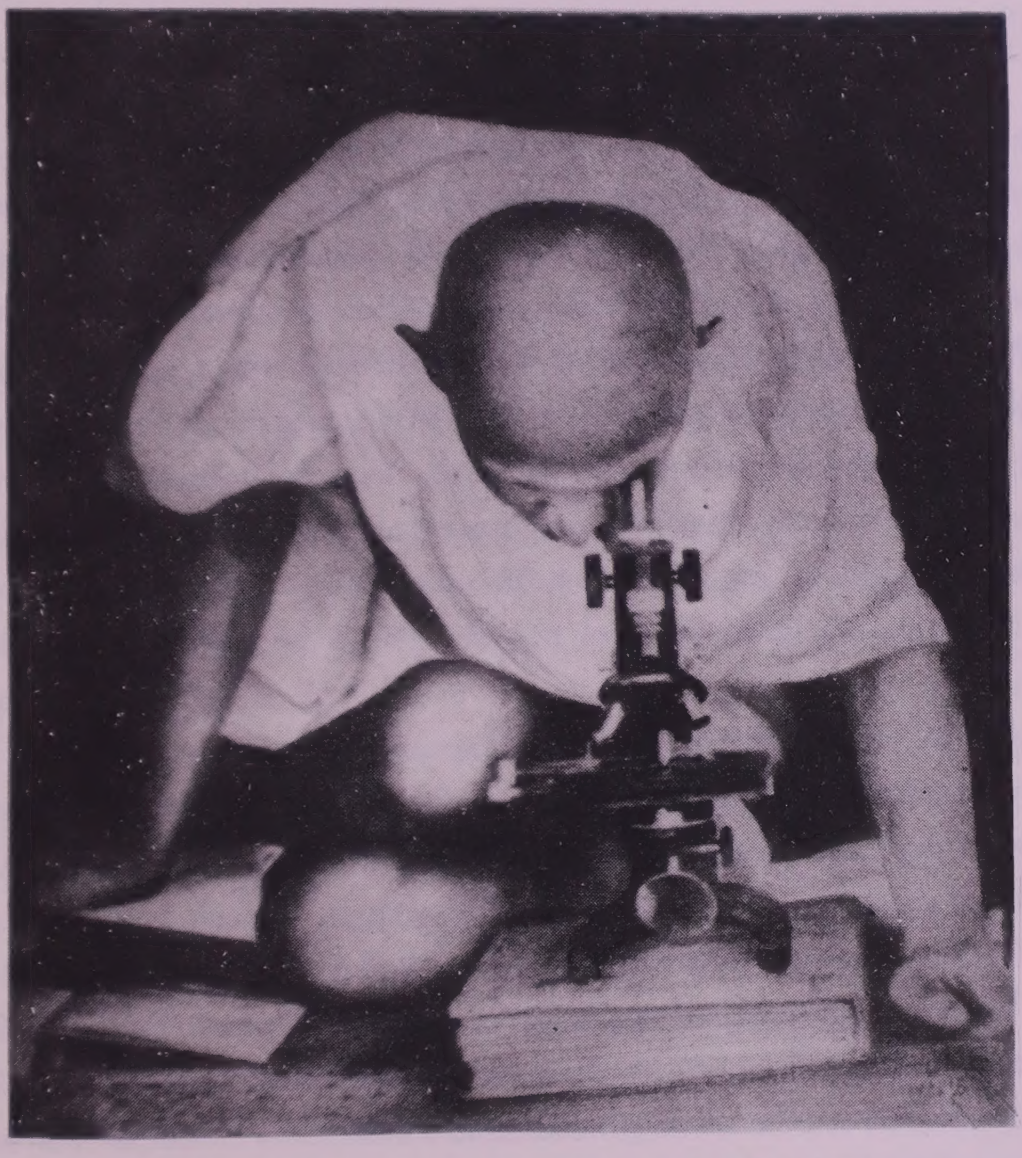
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IN PURSUIT OF KNOWLEDGE AND TRUTH

Foreword

The last decade has seen tuberculosis emerging as the leading killer of all the infectious diseases. It kills more adults each year than AIDS, malaria and tropical diseases put together. One third of the world is already infected with the bacillus and by the turn of the century 300 million more would be added. With every tick of the clock someone in the world gets newly infected. The co-existance of the disease with HIV and the emerging multidrug resistance has further compounded the issue. Estimates show that the decade of 1990-99 will see 88 million new cases of tuberculosis the world over and 8 million will be attributed by HIV co-infection; 30 million will die, of which almost 3 million will be attributed by HIV infection. India will contribute one fourth of the total load.

The best way to prevent the spread of tuberculosis is to educate the people and propagate awareness and awakening among all connected with it. Surveys conducted by the Foundation for Research and Community Health, Bombay have clearly brought out the ignorance and complacency that has infilterated into our system both in the Public and the Private sector. The CME was therefore conceptualised to cover the recent changes in the scenario of the disease. On one hand we observe the rapidly changing trends in demography, the new risk factors which have a direct bearing on the adverse outcome of the disease, while on the other hand research has brought in methods for rapid diagnosis, newer drugs to overcome multidrug resistance and effective programmes of drug delivery to ensure proper intake. All these changes in epidemiology, diagnosis, treatment and control need to be communicated to all but each of these topics is so vast that it could be a CME by itself. Efforts have therefore been made to cover important issues which are either emerging as public health problems or have recently come under the cloud of controversies or have surfaced as newer modalities in diagnosis and

treatment. I hope everyone who gets hold of this compendium based on the CME will find something of their interest in it.

The control of Tuberculosis which was at one stage an imminent reality, has now receded a thousand miles. However, if all the inhabitants of this global village determine to put forth atleast their first step then the journey towards its control may yet become a reality, - "Journey of thousand miles begins with the first step taken".

I am grateful to the Medical Council of India for providing me the opportunity to hold the CME and to invite the foreign faculty from USA and UK. I am also obliged to them for providing finances for bringing out this publication.

To the British Council Division, Mumbai I would like to express my gratitude for sponsoring the travel of the faculty from U.K. I am also obliged to the Ministry of Health and family welfare for providing the timely Grant in Aid.

I take this opportunity to thank Dr. PRJ Gangadharam, Dr. J.L. Stanford, Dr. A.K. Chakraborty, Dr. J.M. Grange and all the guest faculty for their contributions towards the CME and the proceedings.

I am deeply indebted to Dr.Sushila Nayar, Director, Mahatma Gandhi Institute of Medical Sciences, who with her untold dedication (which is reflected in her article) inspired and initiated me into this challenging field of tuberculosis. Without her constant encouragement and guidance we would not have been able to bring out this collection.

Pratibha Narang

MESSAGE

The Department of Microbiology, Mahatma Gandhi Institute of Medical Sciences, Sevagram, Wardha and its indefatigable Head of Department deserve to be congratulated for organising an International CME programme on tuberculosis at Wardha. Nothing could have been more timely, as well as crucially important at the present juncture. WHO has lately declared tuberculosis to be a global emergency. And, though the disease has continued to be a public health problem in the developing world, its resurgence in the developing countries, after having been brought down once, has presented a new challenge. The newly emerged threat is even bigger because of the associated human immuno-deficiency virus disease (AIDS) and multidrug resistance (MDR-TB) in tubercle bacilli.

WHO had been insisting on the confirmation of the diagnosis of tuberculosis, by demonstrating the presence of tubercle bacilli in patient's sputum, body discharges/ fluids or tissues, before starting chemotherapy, since the 1960s. All the unconfirmed cases were to be regarded as suspected cases. The profession, however largely continued to be mesmerized by X-ray diagnosis, sometimes to a great advantage but mostly resulting in overdiagnosis and overtreatment. Perhaps because of this reason, microbiology has played a secondary supportive role, if and when called upon to do so, in most places, till lately. However, the recent mass of scientific evidence favouring the crucial importance of the sputum positive case, especially the smear positive one, and this being made the cornerstone of the revised strategy of the National Tuberculosis Programme has spelled out the strategic importance of the Microbiology laboratory.

By organising the International CME on Tuberculosis, the Microbiology Department of the Mahatma Gandhi Institute of Medical Sciences has done what was long overdue. If the control of tuberculosis is to be achieved, through first dealing with the sputum positive cases, then microbiology has to come forward to assume leadership. Since the 1960s, tuberculosis control has evolved into one of the few Medical Sciences that are truely multidisciplinary, use multipurpose workers, and are truely integrated with the general health services. Therefore, NTP welcomes this initiative: wishes the CME all success; and expects rapid progress made-away from over diagnosis and over treatment which are fairly widespread now-towards correct diagnosis and proper treatment.

(Dr. D.R. Nagpaul)
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CHALLENGE OF PULMONARY TUBERCULOSIS

Sushila Nayar

I became interested in pulmonary tuberculosis in 1950, when I took charge of Badshah Khan Hospital at Faridabad soon after my return after studies abroad from June 1948 to Oct. 1950. Faridabad had refugees population from N. W. F. P. and they had great respect for Khan Abdul Gaffar Khan, the Frontier Gandhi . Hence the Hospital at Faridabad was named after him. It was housed in an old building to start with , but a new hospital was constructed later on along with the new township.

There were 6 cottages with two rooms each for Tuberculosis patients, but there were many more patients among the refugees. The families lived in crowded single room tenements and there were no facilities for home isolation of even a rudimentary type of sputum positive cases. It worried me. I went to Delhi and met the Chief of the Silver Jubilee Tuberculosis Hospital Dr. R. Krishna, who was a friend of my eldest brother Pyarelalji. Dr. Krishna agreed to come to Faridabad every Sunday to see my tuberculosis patients and advise me. Lady Mountbattan had been good enough to give me a station wagon with a driver which enabled me to commute between Delhi and Faridabad as and when necessary. I sent the vehicle for Dr. Krishna every Sunday morning and he spent half the day at Faridabad . We felt that sputum positive cases should not be living with the families in those single room crowded tenements and put up tents in the mango garden to isolate the infectious patients. Thus I soon had a 50 bedded sanatorium there.

I used to examine the sick nurses myself. One day I went to see a

staff nurse who was down with cough and fever-flu as they called it. As I stood by her bedside, she coughed and brought out sputum. A yellowish granule in that sputum looked very suspicious to me . I asked the nurse to bring me a slide. I picked up that granule, went to the laboratory , stained the slide myself and put it under the microscope. It was full of tuberculosis bacilli. After that I sent her sputum to the laboratory several times. It was always reported negative. If I had not prepared and examined the first slide myself , I would have considered that first report to be unreliable and discarded it. As things stood I put her on antitubercular treatment and put up a separate tent for her in the mango grove. She made a rapid recovery and later devoted her life to work for the service of Tuberculosis patients.

I felt we should examine all the refugee population of Faridabad and I went to see Dr. B. K. Sikand the Chief of the NewDelhi tuberculosis Centre.Dr. Sikand told me of his scheme of domiciliary treatment of tuberculosis patients which was prooving very effective. I invited him to Faridabad . He was having a Mass miniature radiography machine and arranged to examine the whole population of Faridabad. We formed Health Committees in each sector of the township and arranged the examination of all the population in each sector. Dr. Sikand used to come every Sunday with his team to carry out the chest examination of all the township men, women and children. Then he would lunch with me and go back. He would often talk of his experience of tuberculosis control in the various wards in Delhi. He told us how the people had been educated to organise care and after care committees. They collected funds for buying medicines for those patients who could not afford to buy them. The Government did not supply free medicines in those days. They also collected money to help the needy ones financially during convalescence and later in finding suitable work for them to enable them to support themselves.One day Smt. Ashadevi Arya Naikam was with me for lunch. After he left, she said to me "Sushila, who was that man? He made romance out of tuberculosis." I told her about his work in Delhi. He had a big map in his side room on which he had put a pin whereever a case of Tuberculosis was and he called it his 500 bedded Sanatorium. He was treating and keeping a track of every case.

In 1952 I fought elections from Delhi, was elected and became Health Minister in Delhi State. I decided that the problem of tuberculosis must be tackled in Delhi. I therefore, called a meeting of leading Tuberculosis experts and we divided Delhi in four zones with New Delhi Tuberculosis Centre, Ramkrishna Mission Tuberculosis Clinic, Peeli Kothi Tuberculosis Clinic and Silver Jubilee Tuberculosis Hospital and put the chief of each incharge of each zone. They were to organise domiciliary treatment of tuberculosis in their areas. But there were some very ill patients who needed institutional care.

The refugees in Delhi had become aware of the danger of tuberculosis and they would carry a patient and leave him or her at Rajkumari Amrit Kaur's door or at my door or under the Minto bridge. It was very distressing. The beds in the S.J.Hospital were all full. I decided that we must do something for these patients who were left by their relatives to die.I had a saving of about 75000 rupees in December and I decided to put up some barracks in the S.G.T.B. Hospital to provide shelter for the patients turned out by relatives to enable them to die in peace. I felt we could have 125 beds in this way, gave 25 beds to each zone entrusted with domiciliary care clinic for seriously ill patients who needed hospitalisation and 25 for the uncared for patients to enable them to die in peace. When we took the proposal of putting up the barracks, Govt.of India experts put up stiff resistance. It was too cold in winter for patients to stay in barracks in Delhi they said. I said Faridabad was only 18 miles away and they survived in tents there and most of them recovered. The Minister of Housing S.Swaran Singh called a meeting and at last my proposal was accepted.

To our surprise many of the patients who we thought were about to die, began to improve after hospitalisation. Ultimately we had to increase the bed strength of S.G. Tuberculosis Hospital by nearly 500 beds. Some quarters built for refugees in an open area of the hospital were converted into the hospital. But I knew, as did the Government advisers that this was no answer to the problem of Tuberculosis.

In 1957 I entered Lok Sabha from Jhansi and in 1962 I became Union Health Minister in Pandit Jawaharlal Nehru's Cabinet. I

visited the National Tuberculosis Institute at Bangalore and saw how they were trying to extend Tuberculosis treatment facilities to sputum positive cases at the Primary Health Centres. All that was needed was a good microscope and a well trained technician to take care of sputum in positive cases by examining the sputum carefully and diligently and putting sputum positive cases on treatment. I also visited Calcutta and saw Dr.Sen's domiciliary treatment of Tuberculosis which was running with great success.

At Madras I saw the Tuberculosis Research Centre which was proposed to be closed down by the Ministry of Health. I felt it should continue and become a referal centre for problem cases regarding diagnosis and treatment and at last this was agreed to. The centre was handed over to ICMR after some time.

In the Health Ministry's Annual Conference we discussed the matter thoroughly and worked out a scheme under which there would be a tuberculosis clinic in every district and a Tuberculosis referal and demonstration centre in every State to help and guide the officers incharge of Dist Tuberculosis clinics. The N.T.I. at Bangalore and Tuberculosis Research Centre, Madras were to function as referal centres and guides for these state research demonstration and treatment Centres. The best demonstration and referal centre was working at Agra under Dr.Mehrotra and I sent several other State officials there to get inspiration. Many states did quite well but several of them were slack. The idea was to diagnose and put on domiciliary treatment all the Tuberculosis patients with positive sputum as quickly as possible. With proper treatment their sputum became negative within a few weeks and thus the pool of infectivity in the community was reduced. New drugs along with I.N.H. proved very helpful and we began to hope to win the battle against pulmonary tuberculosis. Unfortunately we have not succeeded over the four decades since we took up the campaign. There are various reasons for the failure.

In 1969 the M.G.I.M.S. was born and I felt that we would take up the problem of tuberculosis in Wardha District. The I.C.M.R. was kind enough to give us financial support and the Department of Microbiology and Community Medicine together worked out a scheme of Tuberculosis control which gave us much hope and

encouragement. We collected the sputum from symptomatic subjects and examined it under the microscope and also cultured it. We found that the culture gave the most effective response in detecting cases, even more than radiography. We also gave treatment to over 1200 cases in the field and examined their sputum at regular intervals. It was heartening to see that new cases became sputum negative in less than 2 months with biweekly treatment of I.N.H., Rifampicin and Pyrazinamide, followed by INH and Rifampicin for the next 4 months. We continued to check the sputum and found that the response at the end of the treatment of the new cases was 95-97% which was very satisfactory. The chronic cases took 6 to 8 months with only 60 to 70% success rate. But even that is not insignificant.

We did a sample survey on a section of the treated population after a year and found the transmission rate had been considerably reduced. Death rate had also come down. I feel the only way to tackle the problem of increasing drug resistance in cases of pulmonary tuberculosis is quick and systematic treatment of the entire population with effective drugs through District tuberculosis clinics. The sooner it is taken up the better for the country. To that end the district Tuberculosis clinics and demonstration and training centres at state level should be revitalised and their links with N.T.I. and Madras Tuberculosis Research Centre renewed and strengthened. We need men and women with vision and determination to tackle the problem and overcome it. I feel it is time for action and not mere research projects. Medical Colleges should be encouraged to link up with a few surrounding district tuberculosis clinics and strengthen them as also derive experience and strength from the field work of these clinics.

There is much talk of the increased danger from AIDS combined with tuberculosis. AIDS is a problem which requires this country of ours to go back to its moral and ethical values in order to save its youth from this deadly disease. There are no short cuts and no compromises in this regard so far as I know.

Chemotherapy Of Tuberculosis - Promises, Problems And Challenges.

Pattisapu R.J. Gangadharam

Tuberculosis is an age-old disease known to mankind for centuries. It had always been known as the most serious and often fatal disease. Most of the earlier attempts to cure the illness aimed at fortifying the host's resistance by good nutrition, extensive bed rest and healthy and well ventilated environment, factors now proven to be playing a very minor role, if any. By far the most significant in curing the disease is **Chemotherapy** which could be developed and introduced only after the discovery of tubercle bacilli and methods of in vitro cultivation and more importantly animal models, all of which were initiated by Robert Koch at the turn of the century. In a short span of less than 30 years-- starting from the discovery of streptomycin in 1944 to the discovery of rifampin in 1970-- about a dozen drugs were introduced with proven chemotherapeutic activity against tuberculosis. These drugs are classified as bactericidal or bacteriostatic, first-line or second-line, initial treatment or re-treatment drugs based on their activity and the purpose they are used. The first line drugs which are bactericidal are: isoniazid, rifampin, pyrazinamide, streptomycin and bacteriostatic ethambutol. These drugs are the ones used in the primary attack against tuberculosis in the so-called short course chemotherapy of tuberculosis. It is surprising to note that no powerful anti-tuberculosis drug has been introduced since 1970. Some of the possible reasons for this are discussed later.

The proper uses of the drugs in the most useful drug combinations have been evolved following several pains-taking but meticulously conducted controlled clinical trials undertaken in many developing countries, particularly India, Hong Kong and several

African countries. Thus the domiciliary treatment dispensing away with the costly and unnecessary sanatorium treatment, intermittent chemotherapy, and now the short-course chemotherapy-- all were contributed by developing countries only.

In this connection, it is worth paying a great tribute to our **Motherland** which has made significant contributions to the World literature in tuberculosis. Besides the historic Ayurvedic and other ancient literature which consists of the teachings of Charaka, Shusruta and others, modern methods of treatment of tuberculosis have also been introduced by India. Thus the home treatment, intermittent administration, the National Tuberculosis Program etc. to name a few have their origin in India. All these have become the globally accepted methods and are followed by the rest of the world with great success. Even the present-day philosophy of Directly Observed Treatment (DOT) which has been introduced by the Centers for Disease Control (CDC) or the Directly observed Treatment Short-course (DOTS) which is endorsed by World Health Organization (WHO) have their origin in India. Nearly 30 years ago, this concept was introduced in Madras as Supervised chemotherapy of tuberculosis; this is simply re-phrased as DOT or DOTS. Interestingly the picture on the cover page of the WHO pamphlet which is highlighting DOTS, showing the swallowing of pills by a patient in presence of a nurse is from the 1964 Madras study! I have discussed all these aspects in greater detail in a recent presentation entitled "Indian Contributions to Tuberculosis Treatment and Research". Besides myself several world authorities like Gzybrowski, and Mahler mentioned several times that the world owes a lot of gratitude to India for the modern approaches of treatment of tuberculosis.

The powerful regimens using the bactericidal drugs have facilitated the rapid and almost complete (>90%) cure rate of patients even with advance disease. Definitely these regimens prevented the death of patients due to tuberculosis. In the US, an annual reduction of the incidence and prevalence of 10% was seen regularly up to 1984. Based on this encouraging trend, epidemiologists and tuberculosis authorities, particularly in the US, projected a target eradication date of the year 2000, which was

subsequently revised as 2010. This sort of projected optimism resulted in premature slackening of efforts and drastic neglect of the disease. Unfortunately and surprisingly, even the developing and under developed countries, India included, have diminished their concern over this disease. In these countries, most of whom had no backing of epidemiological data, such a diminished interest in this disease was created, perhaps, due to the closure of sanatoria and shifting the treatment of tuberculosis to out-patient setting. It was not appreciated that the closure of sanatoria was not due to the reduction of the prevalence of the disease but that equally effective treatment can be achieved on an ambulatory basis.

The downward trend in the prevalence of the disease and the associated enthusiasm were short-lived, however. At about the middle of the last decade, the prevalance rate stopped declining, which was soon followed by a rapid increase. In a short span of 5 years, there were about 70,000 new cases discovered in the US. This is an alarmingly great increase for this country. More unfortunately, most of these new cases are infected with multiple-drug resistant tubercle bacilli, indicating that the available drugs will not be useful. *Added to this, another international health tragedy, the Acquired Immune Deficiency Syndrome (AIDS) is adding significantly to the seriousness of the problem.

It has been shown that AIDS patients with tuberculosis have only few months to survive, if rapid therapeutic intervention is not implemented. For these reasons, the WHO has declared tuberculosis as **Global Emergency**.

Under this emergent situation, naturally, the number one priority would be to discover and develop new drugs. In fact, several groups of scientists are seriously working with this objective and many drugs are evincing great promise of therapeutic success. One such compound which has been discovered by the author and named by the National Jewish Hospital and Research Center, Denver as **Gangamicin** is being actively pursued by the

*The author has dealt with the problem of Drug Resistance in Tuberculosis in another lecture given in this Conference. He has also written a Text-book and contributed chapters in several other books on this subject. It is also gratifying to note that the Host Institution, particularly Dr. Sushila Nayar and Dr. Pratibha Narang, are actively involved in several studies on this problem, particularly those dealing with the rural population.

author and others. *In contrast to the realization of the urgent necessity of developing newer drugs, a serious problem exists, in that no major pharmaceutical industry is ready to get involved; this is mainly due to financial reasons. It seems that it costs around 200 to 250 million US Dollars to develop a drug, while the Global market, not considering the discounts negotiated by international agencies like the WHO, and UNICEF will be only 100 to 150 million US Dollars! This perhaps is the major reason that no new drug has come out since the Seventies.

The alternative approach, in such a situation, is to try to harvest the maximum benefit from the existing drugs. Such thinking was voiced by Wallace Fox as early as 1972. Experts involved in diseases other than tuberculosis are also entertaining such a philosophy. Assuming that the failure of the existing powerful regimens is due to non-compliance on the part of the patient in taking the prescribed drugs, CDC in the US and the WHO have launched very vigorous programs of DOT, previously called as Supervised treatment. The various field studies conducted in India under the auspices of the World Bank, I am told, are achieving good success rates. In this connection, it is also heartening to note that our host Institution under the stewardship of Dr.Sushila Nayar and Dr.Pratibha Narang produced very high (>95%) cure rates with such treatment policies even under very difficult rural situations. On the other hand, a recent report from the Madras Centre reported a drastic failure of the short course chemotherapy under field conditions.

Careful scrutiny of the success results from Sevagram on the one hand and the failure results from Madras and perhaps from other places bring up several intriguing points on the problems, approaches and challenges of chemotherapy of tuberculosis. While meticulous allegiance to the protocols and devoted scrutiny of drug delivery and drug consumption were ensured in the former, only letter writing was used to achieve compliance in the latter. Field application of chemotherapy also brings up several documented and undocumented problems. In many Centers, adequate supplies of the necessary durgs are not always available. Extensive bureucratic and administrative problems often delay in purchases or stocking

*The author has presented another lecture on "Newer Drugs for Tuberculosis" in this Conference

adequate amounts in the pharmacy. It is not infrequent to hear that the pharmacy has only one of the multiple drugs prescribed. The patient is required to purchase the other drug(s) on his own. Being poor, he normally cannot do so. It is also to be noted that some conscientious individuals in the health administration channel the drugs from the institution to black market. In many cases, the institution's working hours are not convenient to the patient, who cannot easily forego a day's wages to wait at the clinic. All these and for many other stresses the patient is not to be blamed. This aspect has been stressed by me in another article.

The reasons for non-compliance of patients in India and perhaps from other developing countries are different from those of developed countries like the US. In the former, the non-compliance pattern is generally created not by the patient *per se* but largely by the health delivery system. These aspects are excellently brought out by Dr.Pamra. The patient in our country is, by and large, aware of the seriousness of his disease and would definitely like to get cured. The greatest challenge facing us therefore is to correct every loophole in the health delivery system.

Recognizing that daily supervision of drug intake is difficult, if not impossible, we have been attempting to develop depot drug system whereby a single administration can ensure adequate drug levels for a month or more. By doing so, we can reduce the patient visits and other clinic activities and achieve greater success; such an approach will also reduce drug costs and toxicity.

Finally, let me repeat that success in treatment of tuberculosis can be achieved, as the many studies from the host Institution have shown. Let us not point out our finger at the patient showing his faults; let us remember that by doing so, three figures are directed towards us! As Dr.C V.Ramakrishnan has stated that we should not be involved in policy making and protocols only. The success lies in correct, prompt and proper implementation. After all, this is the noble philosophy of Mahatma Gandhi whose legacy is perpetuated in this Noble Institution.

The Microanatomical Nature Of The Tuberculin Reaction

John M Grange

The tuberculin test is among one of the most widely used diagnostic tests world-wide, yet it is certainly one the least understood. Part of the reason for this lack of understanding is due to the complexity of the immune mechanisms in tuberculosis and part is historical.

The long and convoluted story of the tuberculin test began with attempts by Robert Koch to develop a cure for tuberculosis in 1891.[1] During his extensive experiments, Koch observed that guinea-pigs sensitised by infection with the tubercle bacillus developed a characteristic tissue-necrotizing dermal reaction if re-challenged in the skin with tubercle bacilli about one month after the sensitizing challenge. Interestingly, this reaction led to elimination of bacilli from the injection site and the local skin ulcer healed. It was then shown that this reaction, later termed the Koch phenomenon, could be elicited by injection of a bacteria-free, evaporation-concentrated filtrate of the medium in which tubercle bacilli had been cultivated. Koch used this culture filtrate, which he termed Old tuberculin, as a remedy for tuberculosis and, while there were some claimed successes in the treatment of cutaneous and laryngeal disease, patients with pulmonary lesions generally showed no improvement, some did worsen and a few died of severe hypersensitivity reaction ('tuberculin shock').

This episode, which almost ruined Koch's reputation and career, would probably have been swept into oblivion, had it not been for the extensive studies of an Austrian physician, Clemans von Pirquet, whose principal research interest was dermal reactivity to antigens.

Indeed, the skin-prick test system in widespread use in the diagnosis of allergy were pioneered by von Pirquet and still bears his name. Furthermore, he introduced the words *allergy* and *anergy* (from the Greek *allos ergos*, alterted energy, and *anergos*, lack of energy)and he postulated that there was a suppressor, as well as an effector, arm of the immune system.

Among the many antigens studied by von Pirquet was Old tuberculin, which he applied by placing a drop of concentrated material on the skin and making a scratch through it.[2] He undertook an extensive evaluation of this test on the population of Vienna and showed that many persons, not just those with active tuberculosis, gave positive responses and that the proportion of the population giving such responses increased with age. Subsequently, he established that the age-related incidence of positive tuberculin reactivity correlated very closely with the incidence of healed tuberculous lesions found at *post mortem* examination.

During these studies, von Pirquet made two observations which led him to draw conclusions on the relations between tuberculin reactivity and protective immunity.[3] The first was that patient with healed tuberculosis usually reacted to tuberculin but those with advanced, progressive disease, including miliary disease, usually did not. The second was that measles, a condition known to predispose to active tuberculosis in children, caused a temporary suppression of tuberculin reactivity. From these observations, and possibly influenced by Koch's work a decade earlier, von Pirquet concluded that tuberculin reactivity was an indication of a protective immune response in tuberculosis. This conclusion seemed very reasonable and logical at the time but it generated great controversy from that time on.

Indeed, von Pirquet's statement became one of the many dogmas that has underpinned thinking on tuberculosis ever since. Until very recently, for example, it was very widely held that the degree and duration of protection afforded by BCG vaccination was related to the intensity of the tuberculin response of those vaccinated. In fact, von Pirquet's claim was challenged fairly soon after it was published. In particular, American workers demonstrated that infected persons developing small tuberculin reactions often remained

healthy but those with large reactions were more likely to develop overt disease.[4] This has been confirmed in many subsequent human and animal studies.[5,6]

Before expounding on the relation between tuberculin reactivity and immune responses in tuberculosis, it is necessary to look at the nature of the reaction itself and the antigen used to elicit it. As mentioned above, Koch's Old Tuberculin is a culture filtrate concentrated by evaporation. It thus contains many components of the tubercle bacillus including proteins, carbohydrates and lipids as well as substances derived from the culture medium. The original material, which caused some non-specific reactions, has generally been replaced by Purified Protein Derivative (PPD) which is prepared by precipitating proteins from tuberculin by the addition of acetone. Koch also produced 'New Tuberculin' by grinding whole tubercle bacilli to release their cytoplasmic proteins but this manufacturing process was rarely used until Stanford and his colleagues prepared skin test reagent from a wide range of mycobacteria by ultrasonic disintigration, filter-sterlization and dilution to a standard protein concentration.[7] New tuberculin is a much 'cleaner' reagent and induces less non-specific inflammation than PPD.[8] It also appears to permit a clearer differentiation between the qualitatively different types of tuberculin reactivity described below.

The antigens in mycobacterial skin test reagents, however prepared, are divisible into three groups: those unique to the tubercle bacillus, those shared with other species of slowly growing mycobacteria and those commom to all mycobacteria.[9] The presence of the latter two groups of antigens are responsible for cross-reactivity on skin testing and there have been many unsuccessful or at best partly successful, attempts to remove these by various forms of physical or chemical fractionation. Also, much fruitless efforts has gone into finding which component, or components, of tuberculin are actually responsible for skin test reactivity. Almost certainly, many components are involved. One fascinating point that has emerged is that the antigen within a tuberculin reagent responsible for eliciting the skin test reactions differ from person to person. Thus it has been shown that healthy person infected by the tubercle bacilli react to the common, as well as species-specific antigens,

whereas patients with active tuberculosis or leprosy usually show diminished or absent reactivity to the common antigens.[10-11] This has important implications for the development of new vaccine and immunotherapeutic agents.

The microanatomical nature of the tuberculin reaction has been clarified by extensive studies, based on biopsies, by Beck and his colleagues.[12-16] Basically there are three major components of the reaction to injected tuberculin. First, there is an inflammatory reaction of the type that would occur if any mildly irritant foreign material was injected into the dermis. This manifest as a clusturing of blood-derivative white cells foci around the capillaries, sweat glands and hair follicles. The proportion of different types of white cells in this foci is the same as in the blood. This inflammatory foci may be very extensive and, in the centre of the tuberculin reaction, they may occupy up to 40% of the dermis. This infiltration is, however, not the cause of the clinically observed swelling and induration occurring after skin testing. Indeed, there is no correlation between the size of the reaction and the intensity of the inflammatory exudate: the latter may be very intense in persons showing no, little or no visible dermal change and *vice versa*.

Secondly, some of the white cells in the inflammatory foci migrate into the interstitial dermis, particularly to the region just below the epidermis. This phenomenon is more specific as more cells migrate in a reaction to tuberculin than to leprosin in a patient with tuberculosis and *vice versa* in a patient with leprosy.

Thirdly, there is the clinically observable swelling and induration. In this context, it is important to distinguish between swelling and induration and to consider how the two evolve. The state of the vasculature in the test site plays a key role in these reactions. Indeed, it is now evident that vasculitis is at the root of a wide range of a immuno-pathological phenomena and that cytokines produced during immune reactions have profound effects on the micro-vasculature. If the evolution of a positive skin test is observed carefully at intervals of time, it is seen to go through several phases: an initial flare which soon fades, a zone of erythema with a central, soft, oedematous swelling observable after 24 hours, followed by firm induration which reaches a maximum intensity by 48 or 72

hours. In some persons, notably those with active tuberculosis but also healthy hospital workers frequently exposed to infectious patients, an erythematous swelling is clearly evident 6 to 8 hours after skin testing. Some workers considered that the initial flare, the early erythema and the late induration represented, respectively, the classical Types I,III and IV hypersensitivity reactions; i.e.an immediate, atopic, IgE-mediated reaction, an Arthus-type reaction due to antigen-antibody complex formation and delayed, cell-mediated, hypersensitivity respectively. Studies in Indonesia have, however revealed that this is not the case: the initial flare is simply due to dermal irritation and the 6 to 8 hour reaction is an accelerated cell-mediated hypersensitivity reaction due to repeated exposure to antigen.[13,17]

There is, however, a very definite difference in tuberculin reactions that, at 48 or 72 hours, manifest as non-indurating, oedematous swellings and those that show distinct induration. These different responses reflect fundamentally different underlying patterns of immune reactivity with relevance to protection and relate to the earlier observations (discussed above) that small tuberculin reactions appear to indicate protective immunity while larger reactions are associated with progressive disease.

The points raised above raises the paradoxical question as to how immune responses can mediate the apparently diametrically opposed effects of protection against tuberculosis and predisposing to progressive disease. This was a question that perplexed the early tuberculosis immunologists and led to a protracted controversy that culminated in a series of editorials in the American review of Respiratory Disease in 1975.[18-20] At that time , the currently accepted theory of tuberculosis immunity was that developed by Mackeness.[21] In this theory, which still in many respects holds true, antigens of the tubercle bacillus are presented to antigen-specific lymphocytes which undergo clonal expansion and secrete 'macrophage activating factor (now known to be gamma interferon) which greatly increases the ability of macrophage to phagocytose and destroy tubercle bacilli. This was a neat, tidy and highly appealing hypothesis but there was no place within it for the phenomenon of tissue-necrotising delayed hypersensitivity. As so

often happens when observable facts do not fit an hypothesis, such hypersensitivity was dismissed as being no more than an exaggerated macrophage-activating protective reaction. Other workers were not convinced and cited a number of reasons why cell-mediated immunity (CMI) and delayed-type hypersensitivity (DTH) are separable and dissociable phenomena. One author made the prophetic statement that the two patterns of reactivity were due to different populations of T cells, or a single population at different stages of maturity.[19]

In the early 1990s, one of the most important discoveries in the whole field of immunology was made. It was clearly demonstrated, first in mice but subsequently in humans, that T helper cells mature along two quite distinct pathways, resulting in so called TH1 and TH2 cells which secrete different cytokines.[22] Furthermore, the cytokines produced by these two cell types tend to inhibit the other pathway of maturation. In particular, interleukin 10 (IL-10) produced by TH2 cells antagonises the TH1 pathway. As a result, immune responsiveness to a given antigen tends to become 'locked in' to one or other of the pathways.[23] In general, TH1 responses induce protective immunity against diseases characterised by chronic intracellular parasitism such as tuberculosis, leprosy, South American trypanosomiasis (Chagas' disease)and various chronic viral diseases including HIV infection. In the latter , progression from asymptomatic infections to AIDS is accompanied by a TH1 to TH2 shift. TH2-mediated reactions are associated with helminth infection in which the parasites are far too large to yield to macrophages but in which gross tissue necrosis, thereby generating a highly unfavourable micro-environment, may lead to death of the helminth. TH2 activity is also involved in production of antibody, particularly IgE antibody which is an effector in a wide range of atopic or allergic reactions.

In the case of tuberculosis, there is now a large amount of evidence that a pure TH1 response is responsible for granuloma formation and non-tissue-necrotising, protective immunity and that this occurs in the great majority of those infected with the tubercle bacillus. A TH2, or mixed TH1 and TH2 response, on the other hand, is the cause of the extensive tissue-necrosis seen in

progressive post-primary tuberculosis.[24] The basis of this very distinct difference appears to be the result of the action of a key cytokine, tumour necrosis factor (TNF). In CMI, this cytokine, which is present in the cell membrane of the activated macrophages, plays a key role in the formation, integrity and the function of the granuloma. In tissue-necrotising DTH, however, a factor of unknown origin but associated with TH2 cell function, renders other cells exquisitely susceptible to killing by TNF. Certain components of the cell wall of the tubercle bacillus triggers the release of TNF from macrophages and this then kills the sensitised cells, thereby contributing to the gross tissue necrosis that leads to , for example, the pulmonary cavities seen in post-primary tuberculosis.

Clearly, the dose of antigen used in the tuberculin test is selected to avoid the induction of gross necrosis, even though this does on occasion occur. On the other hand, careful examination of tuberculin reactions reveals evidences of necrosis, or of conditions predisposing to necrosis.

Initially, the non-indurating and indurating types of tuberculin reaction described above were, respectively , referred to as the Listeria-type and Koch-type as the former corresponds to the protective response to infection by *Listeria monocytogenes* in the mouse and the latter to the Koch phenomenon in the guinea pig.[25] They are now termed non-necrotising and necrotising responses respectively. One problem with this observation was that the clinical distinction between the two reactions was rather subjective.

A more objective technique for distinguishing between the two types of reaction, and of shedding light on the underlaying mechanism of the differences between them, is laser Doppler velicometry.[19,26,27] This is a technique that measures the blood flow in the skin, or more specifically, the sum of the quantity of blood moving in a unit volume of skin and the mean speed at which it is moving. Not unsurprisingly, the blood flow in erythematous tuberculin reactions, and at the erythematous edge of indurated reactions, was considerably higher than that in the normal skin. Under areas of indurations, however, the blood flow is considerably reduced, often to levels much lower than in normal skin.

The combination of increased metabolic activity of skin at a

tuberculin test site due to the inflammatory infiltrate of cells and the relative slowing of blood readily generates anoxia, acidosis and tissue necrosis. Indeed, anoxia and acidosis have been clearly demonstrated in such test sites by the use of suitable probes. Of particular interest is the finding in one study that persons with large zones of induration and marked central slowing of the blood flow had high levels of IgE antibody against antigen of the tubercle bacillus.[19] In view of the role,discussed above . of TH2 cells in induction of antibody, particularly that in the IgE class, this phenomenon provides further evidence that induration and reduced blood flow is a TH2-mediated reaction.

The other aspect of the tuberculin reaction requiring consideration, and which has been mentioned above, is the reduced activity to common mycobacterial antigens seen in patients with active tuberculosis and leprosy. It is also seen in tuberculin positive, HIV positive persons and those in the inactive phase of Chagas' disease.[28 29] The nature and mechanism of this phenomenon is still surrounded in mystery,and its relation between TH1 and TH2 reactivity is far from clear. On the other hand, it is almost certainly of profound significance to protection as there is strong evidence that 'protective antigens' of mycobacteria are to be found among those common to all mycobacteria rather than among the species-specific ones. [This is evident from the fact that BCG protects as well, or even better against leprosy as tuberculosis in some regions and that it also protects children against lymphadenitis due to *M.avium* and other environmental mycobacteria.] One hypothesis is that an important arm of protective immunity against intracellular parasites is the ability to recognise stressed, pathogen-laden cells and to lyse them.[30] Cell stress induces overproduction of so-called heat-shock proteins (HSP) which flag up cells for destruction. The HSPs are highly conserved so that there is much sharing of epitopes between human and mycobacterial HSPs which form an important, and immunodominant, part of the common mycobacterial antigens. Thus suppression of T cell responsiveness to this group of antigens, and by implication to the HSPs, reflected in the pattern of skin test reactivity, probably indicates suppression or loss of a key protective immune response.

As a result of recent advances in immunology, the early controversies concerning the nature of the tuberculin reaction and its relation to protective immunity can now almost entirely be resolved. There is no longer any doubt that protective cell-mediated immunity in tuberculosis and other chronic infections is quite distinct from the tissue nectrotising hypersensitivity reactions that favour progression of disease. Furthermore, the two reaction are mediated by different and mutually antagonistic cytokines and manifest as qualitative differences in the tuberculin reaction. This supports the old claim that small tuberculin reaction (and it is important here to note that these are recorded as small as, by convention, induration rather than oedema and erythema, are recorded) are associated with protection while the larger, indurated, reactions are indicative of a high risk of activation of disease.

All this would be of rather academic interest if it could not be used to predict and evaluate novel approaches to preventive and therapeutic strategies. Clearly, if a therapeutic agent was available to convert the tissue-necrotising DTH reaction into a protective CMI reaction, with the change being reflected in the qualitative nature of the tuberculin reaction, and perhaps restoration of recognition of the common mycobacterial antigens, it would prove of very great value indeed. One such agent undergoing extensive clinical trials is described in the chapter by Stanford in this book.

In fine, the strange phenomenon named after Koch, which had it not been for the insight and extensive work of von Pirquet could well have been forever overlooked, has generated a vast amount of interest ,as well as dogma and conflict, for almost a century. Even now, immunologists have only began to scratch the surface (metaphorically speaking!) of the dermal reaction to tuberculin. In the words of Issac Asimov, in parody of Willam Shakespeare, "One could, me thinks, wring a flood from a damp cloth".

References

1 Koch R. Weitere Mitteilungen uber ein heilmittel gagen Tuberculose. Dtsch Med Wochenschr 1891; 11:101-102.

2 von Pirquet C.Demonstration zur Tuberculindiagnose durch Hautimpfung. Berl Klin Wochenschr 1909;481:699.

3 von Pirquet C.Verlauf der tuberculosen Allergie bei einem Fallevon Masern und Miliartuberculose. Wein klin Wochenschr 1908:21:861-865.

4 Rich AR, McCordock HA. An enquiry concerning the role of allergy, immunity and other factors of importance in the pathogenesis of human tuberculosis. Bull Johns Hopkins Hosp 1929; 44: 273-422.

5 Bothemly GH, Grange JM. The Koch phenomenon and delayed hypersensitivity 1891-1991. Tubercle 1991; 72:7-12.

6 Fine PEM, Sterne JAC, Conninghaus JM, Rees RJW. Delayed-type hypersensitivity, mycobacterial Vaccines and protective immunity. Lancet 1994; 344: 1245-1249.

7 Editorial. New tuberculins. Lancet 1984; 1:199-200.

8 Morely SM, Beck JS, Brown RA, Grange JM, Kardjito T. Occupational exposure to Mycobacterium tuberculosis and cytology of response to skin testing with 'new tuberculin'. Tubercle 1987:68: 71-72.

9 Stanford JL, Grange JM. The meaning and structure of species as applied to mycobacteria. Tubercle 1974; 55: 143-152.

10 Stanford JL, Nyp PM, Rook GAW, Samuel N, Fairbank A. A preliminary investigation of the responsiveness or otherwise of patients or staff of a leprosy hospital to groups of shared and species-specific antigen of mycobacteria. Lepr Rev 1981; 52.321-327.

11 Kardjito T, Beck JS, Grange JM, Stanford JL. A comparison of the responsiveness to four new tuberculins among Indonesian patients with pulmonary tuberculosis and healthy subjects. Eur J Respir Dis 1986; 69: 142-145.

12 Beck JS. Skin changes in the tuberculin test. Tubercle 1991; 72: 81-87.

13 Grange JM, Beck JS, Harper EI, Kardjito T, Stanford JL. The effect of occupational exposure to patients with tuberculosis on dermal reactivity to four new tuberculins among healthy Indonesian adults. Tubercle 1986; 67: 109-118.

14 Beck JS, Morley SM, Gibbs JH, Potts RC, Ilias MI, Kardjito T, Grange JM, Stanford JL, Brown RA. The cellular responses of tuberculosis and leprosy patients and of healthy controls in skin tests to new tuberculin and leprosin. Clin Exp Immunol 1986; 64: 484-494.

15 Beck JS, Morley SM, Lowe JG, Brown RA, Grange JM, Gibbs JH, Potts RC, Kardjito T. Diversity in migration of CD4 and CD8 lymphocytes in different microanatomical compartments of the skin in the tuberculin reaction in man. Brit J Explt Pathol 1988, 69 771-780.

16 Beck JS, Gibbs JH, Potts RC, Kardjit T, Grange JM, Jawad ES, Spence VA. Histometric studies on biopsies of tuberculin skin tests showing evidence of ischaemia and necrosis. J Pathol 1989; 159: 317-322.

17 Gibbs JH, Grange JM, Beck JS, Jawad E, Potts RC, Bothamley GH, Kardjito T. Early delayed type hypersensitivity responses in tuberculin skin tests after heavy occupational exposure to tuberculosis. J Clin Pathol 1991; 44:
919-923.

18 Youmans GP. Relation between delayed hypersensitivity and immunity in tuberculosis. Am Rev Respir Dis 1975; 111: 109-118.

19 Lefford MJ. Delayed hypersensitivity and immunity in tuberculosis. Am Rev Respir Dis 1975; 111: 243-264.

20 Salvin SB, Neta R. A possible relation between delayed hypersensitivity and cell-mediated immunity. Am Rev Respir Dis 1975; 111: 373-377.

21 Mackaness GB. The immunology of antituberculous immunity. Am Rev Respir Dis 1967; 97: 337-44.

22 Mossman TR. Regulation of immune responses by T cells with different cytokine secretor phenotypes. Role of a new cytokine: cytokine synthesis inhibitory factor (IL-10). Int Archs Allergy Appl immunol 1991; 94: 110-115.

23 Bretscher PA. A strategy to improve the efficiency of vaccination against tuberculosis and leprosy. Immunol Today 1991; 13: 342-345.

24 Hernandez-pando R, Rook GAW. The role of TNF in T Cell-mediated inflammation depends on the Th1/Th2 cytokine balance. Immunology 1994; 82: 591-595.

25 Stanford JL, Lemma E. Subjective interpretation of tests with new tuberculin as a measure of BCG efficacy in Ethiopia. Devl Biol Stand 1986; 58: 745-749.

26 Beck JS, Gibbs JH, Potts RC, Jawad ES, Kardjito T, Spence VA, Grange JM. The relation between cutaneous blood flow and cell content in the tuberculin reaction. Scand J Immunol 1989; 29: 33-39.

27 Potts RC, Beck JS, Gibbs JH, Grange JM, Kardjito T, Stanford JL. Measurement of blood flow and histometry of the cellular infiltrate in tuberculin skin test responses of the typical Koch-type and the non-turgid(Listeria-type) in pulmonary tuberculosis patients and apparently healthy controls. Int J Explt Pathol 1992; 73: 565-572.

28 Khoo SH, Wilkins EGL, Fraser I, Stanford JL. Lack of T cell response to common mycobacterial antigens in HIV-infected individuals - consequence or co-factor? IX International conference on AIDS, Berlin June 6-11th 1993. Abstract of PO-A24-0551.

29 Bottasso OA, Ingledew N, Keni M, et al. Cellular immune responses to common mycobacterial antigens in subjects seropositive for Trypanosoma cruzii. Lancet 1994; 344: 1540-1541.

30 Flynn JL, Goldstein MM, Triebold KJ, Koller B, Bloom BR. Major histocompatibility complex class I-restricted T cells are required for resistance to Mycobacterium tuberculosis infection. Proc Natl Acad Sci USA 1992;89: 12013-12017.

Uses of Epidemiology - Tuberculosis Programme in India

A K Chakraborty

Between 1931 & 1968, London used to have several epidemics of Cholera. One such was raging in the Soho area, some where in 1859. It was Dr. John Snow, a London physician, who had succeeded in pinpointing the source to be a well in Broad street, following a process of epidemiological investigations and reasoning. On his recommendation, just the handle of the hand pump attached to the well, was removed. As the people could no longer pump the well water out, it lead to the immediate disappearance of cholera from the area. This is a simple illustration of the power of epidemiology in disease control. It has often been the instance that simple measures, logically developed on the basis of meticulous observation and analysis of disease variables and dynamics, would bring in dramatic results [1]. TB Programme in India is a case in point to demostrate this.

As is well-recognised by now, tuberculosis is an epidemic disease, with its secular curve being more or less similar to that of other infectious diseases [2,3]. Only difference between the two lies in the fact that the epidemic in tuberculosis could last several centuries, instead of several days, weeks or months, as is common for other infectious diseases. The epidemic curve of tuberculosis, like that in acute diseases, also has an ascending limb (phase of spread), a plateau or peak (transitional phase), a descending limb (phase of decline) and an endemic phase. These disease characteristics would differ from phase to phase, enabling discerning epidemiologists to work out the respective age of the epidemic. These would also make it possible to identify aspects of the disease distribution and behaviour, which could be utilised in designing the appropriate and applicable measures to control the disease. In India, the disease characteristics have been analysed and the specific aspects of these have been identified and used as rationale for designing the NTP. Also the epidemiological information has been interpreted to form the hypothesis on the age of the epidemic or the trend.

Uses of Epidemiology - Tuberculosis in India

Presented below are some of the epidemiological features of the disease and their significance, viewed as a rationale for activities under the National Tuberculosis Programme (NTP) with particular reference to the epidemiological situation in India. The likely interpretation of data and inference regarding the epidemiological situation in the country is also given:

Summary of epidemiological observations from : (a) global studies; (b) Indian surveys	Comments/inference on likely trend or epidemiological situation in India	Epidemiological rationale of NTP in India
1	2	3
(1) Tuberculosis is a long term epidemic spanning several centuries (FIG.1). (a) Changes in disease trend over a short span of time are not expected, except when efficient intervention are undertaken as in the Eskimos (hypothetically possible). Transient changes in the epidemic curve may occur, e.g., through war, famine etc., but the trend resumes its natural course after their effects disappear (secular trend) [3]. (b) Two points of observations separated by even 10-25 years or more are so close to each other in the context of the epidemic curve spanning several centuries, that quantitative changes cannot be measured, unless massive (which is unlikely) [4].		(a) Tuberculosis programme should be a long term programme. Vertical programmes not suitable. Case finding/ treatment activities to be carried out as service component of an integrated health care delivery system. (b) No quick change in situation to be expected (c) No all India repeat sample survey at small intervals of 10-25 years called for.

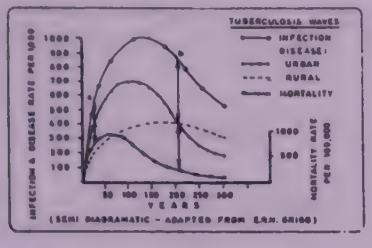
(2) Tuberculosis problem is more or less spread all over the country. Difference in disease prevalence rates between area to area not statistically significant (barring a few exceptions eg., Raichur and urban slums eg., in Calcutta) [5].	Wide spread tuberculinisation has taken place, with the inference that tuberculosis curve is in endemic phase in the country.	Almost a similar and uniform kind of programme is required all over the country. No special programme from place to place was formulated for urban slums etc., considering the infancy of the national endeavour, when launched!
(3) Prevalence rate of case is same for the rural and urban areas [5].	Disease not likely to be in the spreading phase: Probably on the descending limb.	In absolute number, 80% of the total problem is distributed in rural areas (since 80% of the population live in villages). Hence, tuberculosis services should cover rural areas adequately. Emphasis on spread of programme.
(4) About 38% of persons all ages both sexes and almost 70% of males above 40 years of age infected. About 2% are diseased. Prevalence of bacillary cases: 0.4% . Mortality rate: 0.09% (4) See Annexure.	The wide gap between the rates may mean that the disease is on the descending phase (FIG 1.)	Presence of 38% of total persons infected in the community may mean that eradication cannot be conceived as a goal. .
(5) Prevalence rate of infection and disease as well as mortality are more in males than females. (FIG 2)	The disease is beyond the peak, probably in the descending limb of the curve or endemic phase [3].	Surveillance system should be developed to study infection rate in different parts continually, with provision for stratification even within districts. This could serve as effect-evaluation of the programme if performed every 7-10 years, area wise.
(6) There is a decreasing trend in the infection rate of about 2% per year, over a long term observation for a period ranging from 5-25 years or more among children (from longitudinal surveys of NTI).(FIG 3) But the same is not seen in the adjoining areas of Tumkur, Doddaballapur and in the neighbouring state of Tamil Nadu (over 15 years) [4].	Probably represents a secular trend of infection rate. Tuberculosis situation may be on slow downward trend in some areas and not changing in some other areas. It is logical to postulate different epidemiological age from area to area in India, given its vast expanse [6].	

(7) Pulmonary tuberculosis is an adult disease.. Population in 0-19 years (comprising 50% of total population) contain only 7% of total prevalence cases. Remaining 93% of cases are distributed in population aged 20+ years. In the first of the longitudinal surveys, in Bangalore area, the proportion of cases above 40 years in age was around 50% (0-39 : 40+ yrs:: 53: 47), which was in about 20% of population. In another 30% of population, i.e., in those 20-39 years age group, 43% of cases were distributed [7]. (FIG 4)	Proportional concentration of disease in adults may mean that disease is beyond the peak. It is most likely that the epidemic curve is not on ascending limb. Even as the population aged 40+ would contain 50% of cases, the public health significance of young adults of between 20-39 years in age containing 43% of cases cannot be too over-emphasised - as it happens to be the most productive segment in population. Similar is the situation in women, where the disease burden is borne by those in the reproductive age, nearly half of the cases in women being in this age group.	Since cases in adults can be easily diagnosed by simple tools (microscopy) from among symptomatics, disease among relatively articulate adults is possible to be tackled under a public health programme. (Note: 80% of cases are aware of symptoms and 50% are action taking). Had the disease been more prevalent in children, control programme might not have been feasible (Note: Inarticulate children and difficult diagnostic tools would pose problem: hence programme for wide application would have been less feasible.)
(8). Relatively higher and higher concentration of cases in higher age groups has occurred in later surveys as compared to earlier surveys. In later surveys in Bangalore rural areas, about 80% of cases were detected among those in 40+ age group .	May mean that tuberculosis is in declining phase.	Programme interference-efficiencies be augmented to hasten the decline.
(9). Prevalence rates of cases and suspect cases, reveal almost no change in a period of over 20 years from different surveys in different areas [4].	No change is expected over a relatively short period of time in disease rates which are already low. Moreover disease is not in its spreading phase: may be on a descending limb or in endemic phase.	No all India repeat sample survey for disease prevalence may be indicated, as this may not yield much additional information.

10. However, the incidence of smear-positive cases in the community is observed to have dwindled in 23 years in Bangalore rural area from around 65 to 23 per 100,000 [4]	This is commensurate with fall in the annual risk of infection (ARI) from 1% to 0.6%. The ARI in most other areas, except possible for Kashmir, are static over the years. The high ARI with no or small extent of decline below 3% per year, mark out India to be one of the high prevalence countries, together with the sub-Saharan African countries.	Slow downward trend of tuberculosis in the area, along with high transmission, calls for a highly efficient programme.
11. Nearly 10% of all causes crude mortality in the community is contributed by death due to tuberculosis (tuberculosis mortality rate : about 90 per 100, 000) [9].		Tuberculosis is a major public health problem and deserves appropriate priority.
12. (i) Mortality rate decreasing with time from survey to survey, but is still high [9].	i) Disease is on downward trend or in the endemic phase.	Better case-holding should be attempted to save life. Treatment services to be widely distributed through integrated delivery approach.
(ii) Survival rate of cases diagnosed in later surveys were better as compared to in earlier rounds of longitudinal surveys. Paediatricians generally report seeing less of miliary, meningeal or fulminant forms of tuberculosis. Same is the experience of tuberculosis specialists, working in clinics.	ii) Policy of BCG vaccination to younger children might have been effective in revising the incidence characteristics in children, by arresting haematogenous dissemination.	BCG vaccination programme to be continued in younger population (0-1 year or 0-4 years).

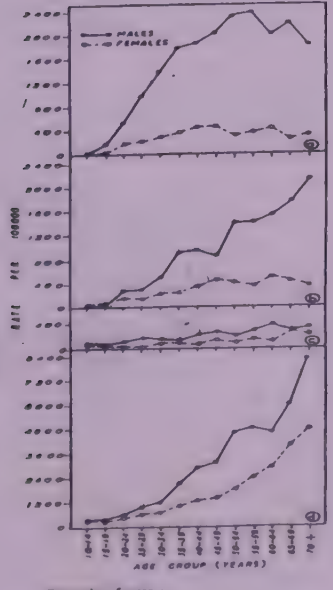
Fig.1. HYPOTHETICAL SECURLAR CURVES OF TUBERCULOSIS EPIDEMIC

(Infection, Disease & Mortality Trends)



SOURCE: ERN GRIGG (3)

Fig.2. PREVALENCE OF DISEASE BY AGE SEX AND METHOD OF DIAGNOSIS

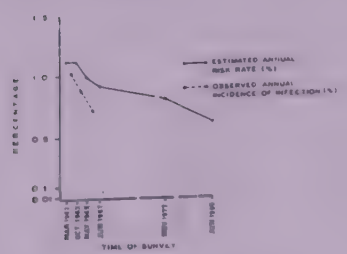


Categories of cases :
a) Culture Pos. on 2
b) Culture Pos. on one specimen only
c) Culture Neg. smear Pos. (3 or more AFB)
d) Abacillary MMR - active (by 2 readers)

SOURCE : BCG. TRIAL (10)

Fig.3. TUBERCULOSIS TREND OVER 23 YEAR PERIOD

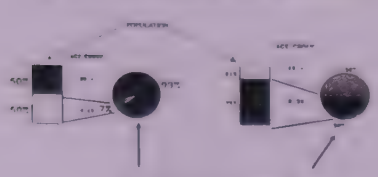
(Bangalore Rural Area)



SOURCE: CHAKRABORTY AK et al (11)

FIG. 4

DISTRIBUTION OF AGE-WISE PREVALENCE OF BACILLARY CASES IN THE COUMMUNITY



TB CASES IN THE POPULATION

References

1 Morris J N Uses of epidemiology, 1967. 2nd Edition (Reprint), L & S Livingstone, edinborough & London.

2 Nagpaul DR Tuberculosis in India - a perspective *J.Indian Med Assoc* 1978; 71: 44

3 Grigg ERN : the arcana of tuberculosis with a brief epidemiologic history of the disease in USA Amer Rev Tub, 1958, 78, 151 (Part I & II), 426 (Part III).

4 Chakraborty A K tuberculosis situation in India: measuring it through time Ind. J. Tuber 1993; 40: 215-255.

5. Indian Council of Medical Research: Tuberculosis in India- A sample survey 1955-58, special report series No 34 ICMR New Delhi, 1-121.

6. Chakraborty AK : Introduction, in : Urban tuberculosis control : problems and prospects, Ed. Chakraborty AK et al, 1995, FRCH Bombay, Pp 1-127 (+ i-ix).

7. NTI Bangalore : Tuberculosis in a rural population of south India, a five year epidemiological study, Bull Wld Hlth Org 1974; 51: 473-488.

8. Chakraborty AK et al: Tuberculosis in a rural population of south India : Report on five surveys. Ind. J. tuber 1982; 29: 153-167.

9. Chakraborty AK et al: Tuberculosis mortality rate in a south Indian rural population, Ind. J.Tuber 1978; 25: 181-186.

10. Tuberculosis Prevention Trial Madras: Trial of BCG vaccines in south India for tuberculosis prevention, Indian J.Med Res 1980; 72 (Suppl): 1-74.

11. Chakraborty A K et al: Tuberculosis infection in a rural population of south India : 23 years trend, Tubercle and Lung Dis 1992; 73: 213-218.

ANNEXURE
PROBLEM OF TUBERCULOSIS IN INDIA
(estimated on 1991 population)

1. Population : 844 million : 726 million in 5+ age (85%)
2. Prevalence of Infection:
   (a) Percent - 38% (all ages) b) more than 50% in 40+ age
3. Prevalence of radiologically active abacillary pulm. TB
   a) Rate - 1.6% b) Number - 12 million
4. Prevalence of positive cases. a) rate - 0.4% b) Number - 3million
5. Prevalence of total cases a) Rate - 2.0% b) Number -15 million
6. New patients arising annually : 2.5 to 3 million
7. New bacillary cases arising annually: 1 million
8. Case fatality Rate : 14% in untreated bacillary cases
9. Mortality
   a) Rate - 50-80/100,00 population b) Number - 300, 000 to 500,000 (Annually)

Tuberculosis And hiv In Mumbai

Pratibha Dalal

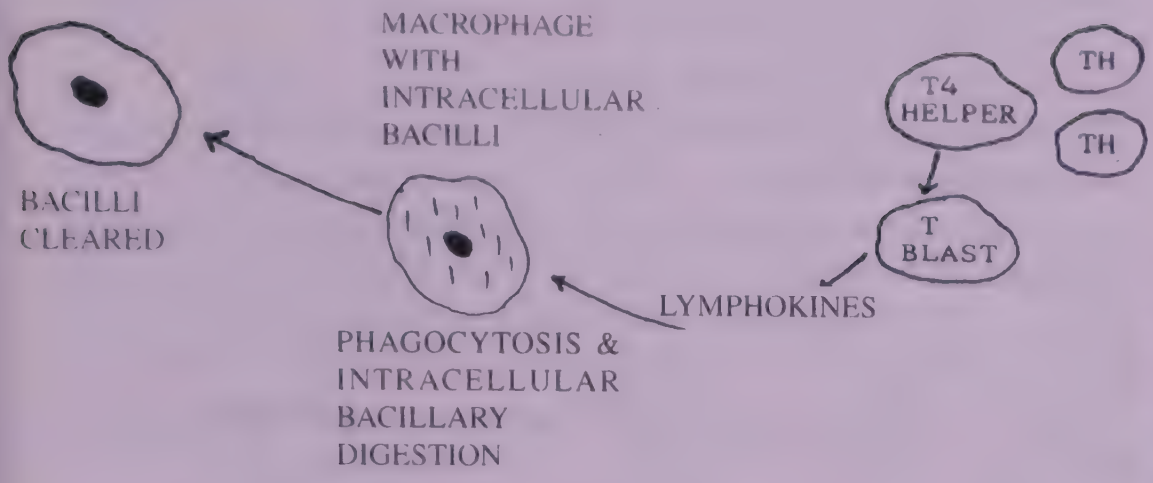
Tuberculosis continues to be a major public health problem in India. Inspite of the National Tuberculosis Control Programme being in operation since 1962, there are no signs of control over Tuberculosis. According to the statistics presented by the Director, National Tuberculosis Institute, Bangalore, about 50% of the adult population is infected with tubercle bacilli , and new tuberculosis cases continue to arise from the already infected pool.

Every year nearly 3 million new Tuberculosis cases arise in the community, out of which about 1 million are sputum positive. The disease is equally prevalent both in urban and rural population.The number of tuberculosis cases is increasing due to the dual epidemic of TB & HIV. The prevalence of HIV infection in India is also on a rise and the number of HIV infected individuals in India are over 1 million. The association of HIV and TB epidemics became evident from the observation in USA that the top five ranked cities for diagnosis of AIDS had also reported the maximum increase in TB case rates.

How does HIV infection predispose an individual in the development of tuberculosis ?

- In an IMMUNOCOMPETENT PERSON during the intial (primary) infection with M.TB, macrophages ingest the organisms and process and present the mycobacterial antigen to T cells. CD4 cells secrete lymphokines and enhance the capacity of macrophages to ingest and kill the mycobacteria. In most people tuberculosis infection is contained and tuberculosis does not develop although small number of dormant bacilli may remain in the body.

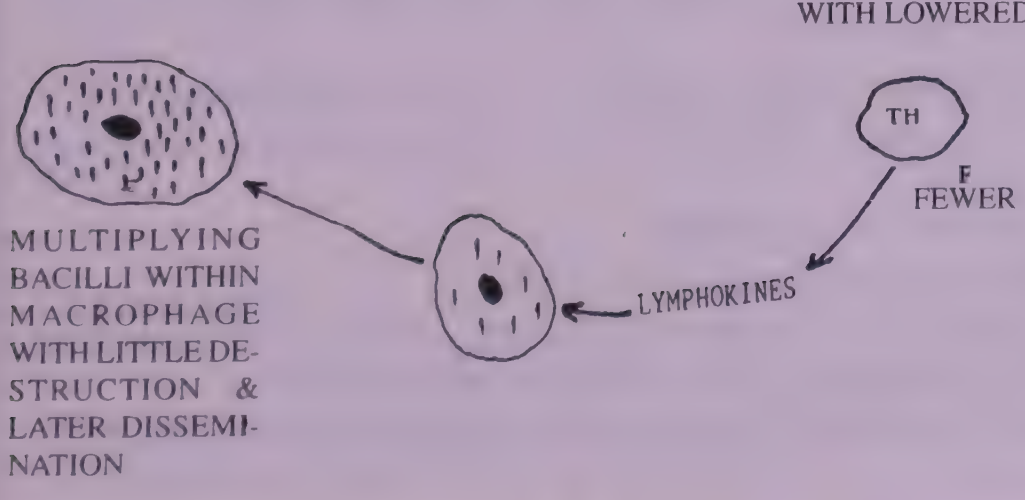
IMMUNOCOMPITANT HOST



Clinically apparent TB develops in approximately 10% of infected patients soon after primary infection or years later (reactivation tuberculosis). These consequences are thought to be due to defects in T cells or macrophage function or both.

The hallmark of HIV infection is progressive depletion and disfunction of CD4 cells coupled with defects in macrophage and monocyte function. Since CD4 cells and macrophages have a central role in antimycobacterial defences, dysfunction of these cells places patients with HIV infection at a high risk for primary or reactivation tuberculosis and also increases the risk of progressive disease from new infection.

IMMUNOCOMPROMISED HOST (HIV INFECTION)



Clinically the two most obvious differences between HIV positive and HIV negative TB are :-

1. The location of the disease.
2. Findings on chest roentgenogram.

- Patients with HIV associated TB are more likely to have extrapulmonary disease, miliary infliltrates, central nervous system disease including tuberculomas and tuberculous meningitis and nonreactive tuberculin skin test than those with tuberculosis who are HIV negative in whom the primary infection usually involves the lungs.
- Chest X ray findings are variable and depend upon the stage of the disease ->

In the early stage of HIV disease, upper lobe infiltrates and cavitation typical of reactivation tuberculosis are commonly seen.

Hilar lymphadenopathy , miliary pattern, pleural effusion and diffuse interstitial infiltrates are commonly seen in those HIV positive patients who have severe degree of immunosuppression.

Due to immunosuppression the patients become susceptible not only to M. TB but atypical mycobacteria also.

Patients with HIV infection with or without diagnosis of AIDS or AIDS related complex have an increased incidence of tuberculosis and therefore there should be a high index of suspicion of TB in patients with HIV infection.

Conversely HIV infection should be suspected in patients with unusual manifestation of tuberculosis.

The research carried out by our department on the corelation of clinically and radiologically diagnosed tuberculosis with HIV from 1989 onwards shows a gradual rise in the incidence of HIV reactivity. (Refer to graph p-37).

Based on these findings, in September 1993, we set up a project to -

- Isolate and identify the strains of mycobacteria in pulmonary tuberculosis.
- Corelate PTB with HIV.
- HIV type prevalent in our sample.

For the study, 350 clinically suspected cases of PTB attending JJH from September '93 to June '94 were selected.

Blood and 3 early morning sputum samples were collected from each patient.

Out of the 350 sputum samples, 110 were positive for Mycobacterial growth. These were then characterized by using the following criteria :

- Speed of growth
- Photoreactivity
- Niacin test
- Catalase test - 68°C
- Nitrate test
- Urease test
- Tween hydrolysis test
- Aryl sulfatase test
- Pyrazinamide test
- P. Nitrobenzoic test

On characterization it was found that of the 110 isolates :

SPECIES	NOS	%
- M. tuberculosis	101	91.82
- M. Kansasii	04	3.60
- MAIC	03	2.73
- M. szulgai	01	0.91
- M. asiaticum	01	0.91

Blood sample was collected on day one from all the 350 patients. Serum was seperated and at first tested for HIV-1&2 antibodies by the indirect microtitre well ELISA. Out of the 350 samples 43 tested positive for HIV-1 &2 antibodies. These 43 samples were then retested by using an immunocomb Bi-spot HIV 1 &2 kit and confirmed by Western Blot. All the 43 sera showed a reactivity to HIV-1 antibodies only.

Out of the 110 mycobacterial culture positive , 25 sera were also positive for HIV-1 antibodies and out of the total 240 mycobacterial culture negative patients, 18 were positive for HIV-1 antibodies.

CORRELATION OF HIV STATUS AND MYCOBACTERIAL RESULTS :-

SAMPLES		HIV +ve(%)	HIV-ve (%)
Total	- 350	43 (12.4)	307 (87.6)
Culture negative	- 240	18 (7.5)	222 (92.5)
Culture positive	- 110	25 (22.72)	85 (72.28)

Of the 25 culture positive and HIV -1 antibody positive, 21 were isolates of M. tuberculosis and 2 each of M. kansasii and (MAIC) Mycobacterium avium intracellulare complex.

MYCOBACTERIAL SPECIES ISOLATED IN HIV +ve CASES :

SPECIES	NUMBER
M. tuberculosis	21
M. kansasii	2
MAIC	2

Thus,

-M. tuberculosis is the most prevalent strain though atypical mycobacteria are also seen.

- 12.4% of the samples tested were positive for HIV-1 antibodies.

Multi Drug Resistance Tuberculosis (MDR- TB)

The reports of multidrug resistant tuberculosis is on a rise especially in association with HIV and has caused great concern owing to the very high case fatality rate.

It is possible that poor patient compliance combined with inadequately funded TB control programme in the age of AIDS may lead to the emergence of increased number of resistant strains. If these spread increasingly in the community, TB may become progressively uncontrollable using currently available chemotherapy.

In India an accurate picture of drug resistance is not available due to lack of MDR- surveillance systems and adequately equipped laboratories. The prevalence of MDR-TB is increasing but reliable data is not documented yet.

Before discussing about drug resistance, there are certain definitions which should be understood :-

Primary Resistance :- Is defined as the presence of drug resistance to one or more antituberculosis drugs in patient who has never received prior TB chemotherapy. (It is caused by infection with drug resistant organisms from another patient who has acquired resistance either due to inadequate chemotherapy or because of infection with primary drug resistant organisms).

Acquired Resistance :- Is defined as resistance to one or more antituberculosis drugs which arises during the course of treatment usually as a result of non-adherence to the recommended regimen or faulty prescribing (This can also be referred to as

secondary resistance).

Initial Resistance :- Is defined as the presence of drug resistance to one or more antituberculosis drugs in a new tuberculosis patient who presents to a treatment centre (This category includes those patients with primary resistance as well as those patients with undisclosed acquired resistance).

Multi -Drug Resistance :- Is defined as resistance to more than one antituberculosis drugs (W. H.O. recommendation).

There are three widely used methods for drug sensitivity testing on L.J. medium.

1. Absolute concentration method
   (Minimal inhibitory concentration).
2. Resistance ratio method
3. Proportion method.

However these methods require atleast one month for the results to be available.

The BACTEC radioactive liquid medium is the fastest way to measure resistance , yielding results within less than 10 days after isolation of the organism but is also the most resource intensive and is not yet routinely available in the developing world.

Luciferase reporter assay, although still in experimental stage, appears to be a promising technique for drug sensitivity testing for MDR-TB.

With the help of restriction fragment length polymorphism (RFLP) another molecular biology technique, one can rapidly categorize patients isolate as drug sensitive or MDR-TB and the drug treatment modified accordingly.

We in our department tried to study the prevalence of MDR-TB in the acquired resistance cases.

A total of 240 patients who were clinically and radiologically diagnosed as tuberculosis and who were defaulters presenting for treatment at the hospital were taken up in the study.

Of the 240 samples processed, 80 (M.TB- 78, M.avium- 1 and M. terrae-1) were positive for culture.

Drug susceptibility testing of these 80 culture isolates was done by the Resistance Ratio Method using the 1st line of drugs - INH , Rifampicin, Streptomycin & Ethambutol and it was found that :-

DRUG SENSITIVITY PATTERN	NUMBER	%	
Sensitive to HRSE	42	52.5	
Resistant to HRSE	12	15	
Resistance to R +H	5	6.25	35
Resistance to R +S	5	6.25	
Resistance to H +S	6	7.5	
Resistance to R	4	5	
Resistance to S	5	6.25	
Resistance to H	1	1.25	

Thus it can be seen that MDR-TB accounted for 35% in our study group.

2nd line of drugs can also be used for the treatment of TB. However these drugs are less potent than 1st line drugs and most cause significant side effects which sometimes warrant their discontinuation. Also majority of these drugs are very expensive and patients are not able to afford these drugs in the developing countries.

A number of new drugs have been tried in the treatment of TB. (These include quinolones, amikacin, rifamycin derivatives, clofazimine, B.lactams and macrolides). Although many of these drugs have been claimed to be effective in vivo and in vitro, well designed randomised controlled trials are not available. Also appropriate dosage of these drugs are not established, they have side effects and their safety during pregnancy has not been established.

References :

1. Khilnani G C , Sharma S K. & Pande J N : Multidrug resistant Tuberculosis Review: The Indian J Chest Dis Allied Sci , 36, 3,(1994) 137-145.

2. Vareldzis B P , Grosset J de Kantor I, et al : Drug resistant tuberculosis. Labortory issues : World Health Organization recommendation Tubercle and Lung Disease, (1994) 75, 1-7.

3. Liza Roy Varghese : C M E Tuberculosis and AIDS. Ind J Tub. (1991) 38, 167 - 171.

4. Rosenheim M: Problems of diagnosis and thesapy. Bulletin of the Internation

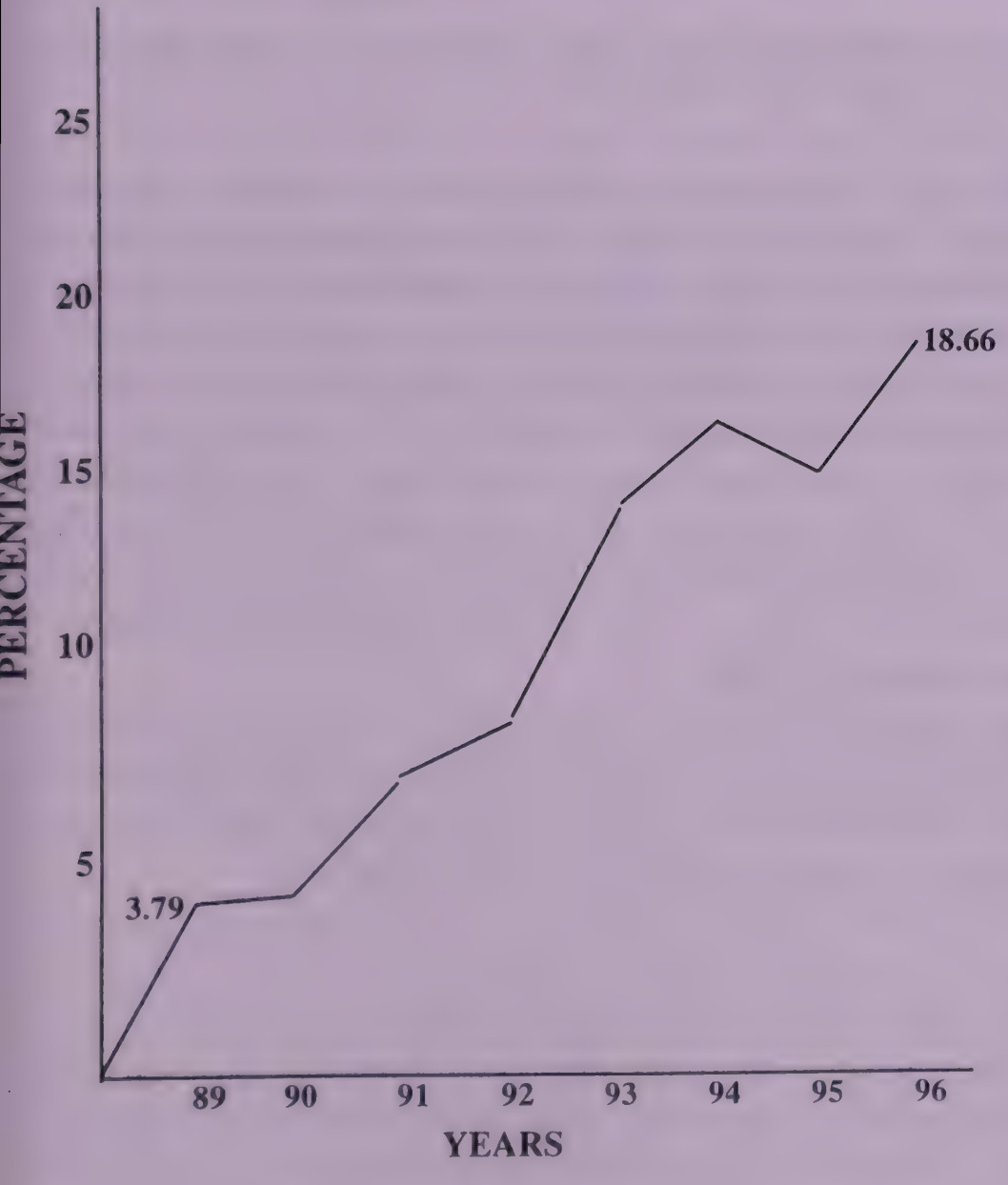
Union Against Tuberculosis and Lung Disease; 65. N.1 (1990), 35 -36.

5. Drobniewski ; FA , Pozniak AL, and Uttley AHC, Review article, Tuberculosis and AIDS ; J. Med. Microbiol. 43 (1995) 85 - 91.

6. Sharma S K ; Pande J N ; Editorial. HIV infection and Tuberculosis ; Indian J Chest Dis Allied Sci. 36 3, (1994), 109 - 111.

7. Mohanty K C , Sundram R M , Nair S. HIV infection in patients with respiratory disease. Indian J Tub (1993) 40: 5 - 12.

DISTRIBUTION OF HIV IN TUBERCULOSIS CASES



Prediction Of Relapse In Pulmonary Tuberculosis An Epidemiological Model

Mrinalini Pathak, A.B. Amle , I.R. Kulkarni , B.R. Maldhure & C. S. Pathak

Relapse is sad unwelcome but distinct occurance in quiescent pulmonary tuberculosis patients. As per recommendation of British Tuberculosis Association, the patient who has been treated for tuberculosis should be kept under observation possibly for rest of his life. This policy places a heavy burden on health services and creates anxiety in patients because of periodic follow up. An estimation of probability of relapse in each case of tuberculosis, successfully treated, will be useful in formulating effective strategy for follow up. The present attempt is undertaken with the idea of developing such epidemiological system, which can estimate probability of relapse.

General Logic of Model

All treated cases of tuberculosis can be categorised into two distinct groups, relapse (R) and nonrelapse(NR), based on whether sputum again shows mycobacterium tuberculosis or not. Thus this becomes the critical criterion for classifying the case.

However, this outcome is affected by the fact that there are other factors which predict this outcome e.g. regularity in treatment, initial extent of disease, etc. If all these factors have some importance, the sum of all these variables can be treated as single variable and critical score will then be mid point of two means of respective groups. However, it is observed by author in previous case - control study of relapses that all these variables do not have equal impor-

tance since Odd's ratio differs for various factors. Therefore in order to get full advantage of co-relation, different weightages have to be assigned to these various factors depending on their individual co-relation with the outcome. The weighted sum of these score is then obtained for two groups separately in which relative importance of these factors are balanced. The weighted mean of two categories thus obtained can be used to obtain a composit variable by assigning weightage to all variables. The method is called discriminant function analysis.

Material and Methods

The data used for construction of this model was extracted from records of cases of pulmonary tuberculosis attending T.B. control and Training Centre Medical College, Nagpur during period March 1984 to December 1986. During this period total 4720 sputum positive cases were detected. Out of these 2509 cases were put on short course Chemotherapy while others were put on conventional regimen. Out of these cases 1321 (52.6%) desired to take treatment from other centres & were referred to other centres for thearpy and follow up while 1188 (47.35%) were treated at the centre which formed the study material.

Relapse was defined as:-

1. Patient was diagnosed as sputum positive case of pulmonary tuberculosis.
2. Treated with appropriate drug combinations.
3. Completed prescribed course of Chemotherapy.
4. Their sputum conversion status remained negative atleast for 6 months from last negative report of sputum examina -tion till the occurance of relapse.
5. Quiescent patient whose disease had bacteriologically relapsed.

Out of 1188 cases of pulmonary tuberculosis 38.5%(457) were lost to follow up for various reasons, 26 patients developed drug toxicity, 21cases migrated to other districts, 15 cases died. Thus total 519 (43.7%) were excluded. 669 cases completed Chemotherapy and were declared quiescent. Total 32 cases (4.7%) met the criterion of relapse. For each case of relapse one case of Pulmonary Tuberculosis which had not relapsed was selected to form non relapse or control groups.

Criteria for inclusion as control were :-

1. Patient was diagnosed as sputum positive pulmonary T.B. case and was treated appropriately.
2. Patient completed prescribed course of Chemotherapy and was declared quiescent.
3. Patient remained inactive since he was declared quiescent.

The clinical profile of the patients :-

No.of sputum positive cases detected	-	4720	
No. of cases treated at DTC	-	1188	(47.35%)
No.of cases dropped	-	519	
Lost to followup	-	457	
Drug toxicity	-	28	
Migration	-	21	
Death	-	15	
No. of cases completing treatment	-	669	
No. relapsed	-	32	(4.7%)

Clinical profile of cases treated :-

Mild	-	62
Mod Advanced	-	614
Far Advanced	-	512
Cavitative Lesions	-	618

7.3% cases had initial resistance to INH while 4.88% amongst relapsed and 2.94% amongst controls were resistant to streptomycin. No case with initial resistance to rifampicin was observed during the study period. 41.46% of relapsed cases and 17.00% of nonrelapsed cases required hospitalisation during treatment period. 44.9% of relapsed patients and 23% nonrelapsed patients had concurrent diseases associated with tuberculosis. The associated illness were diabetes mellitus, enteric fever, cirrhosis of liver, psychiatric disorders malignancies etc. None of the patients detected was HIV positive during this period. Only 10% patients were regular in taking the treatment i.e. did not require single default action while 50.7% were defaulters for more than 60 days. 64% patients were sputum negative in less than 15 days while 22% required more than 60 days for sputum conversion. 17% patients had cleared lesion at

the end of Chemotherapy while 12% deteriorated or had residual cavity (18.28%).

From the records of the patients all the relevant information was extracted on pretested proforma. Odd's ratio was estimated for various risk factors suspected to be associated with relapse and the statistical significance was tested. A risk factor which was having significant odd's ratio indicating strong association with relapse were included in model.

The table I shows the description of the variables along with the numerical coding. Out of 82 records used for construction model 18 were discarded as they had incomplete information. Out of remaining records 20 each for relapse and nonrelapse group were randomly selected for inclusion in the model.

To give some idea of the extent to which relapse and nonrelapse cases differ with respect to the variables, mean of two groups along with means and Standard Deviation in entire normative sample are given in table II.

When two groups are to be discriminated between the discriminant function is essentially the same as multiple regression equation of dummy variable (X10) on predictor variable. Therefore 10^{th} variable is defined to take value 1 and 2 for two groups respectively i.e. each of the patient is normalised sample who did not relapse was given score of 1 while those who relapsed were given score 2. The product moment co-relation coefficient was estimated between each pair of variable taking all 10 variables. The symmetrical matrix obtained thus is shown in table III.

In accordance with multiple regression theory these corelation co-efficient determine 9 linear equations which on solution gives 9 partial regression weights to be applied to 9 predictor variables. These are shown in table IV.

The second column of table IV shows the resulting standard regression weights or beta weights. These indicate relative importance of corresponding variable within 9 predictor variables. These are then transferred into B weights by multiplying beta weight of each variable and dividing each product by standard deviation of corresponding variable. These are shown in column B of table IV.

The next step is taken just as a matter of convenience in order to

have largest weight equal to unity. This could be done by dividing each B weight by 0.543748 which happens to be the highest value (X6). The results are shown in the last column. Using these Vi weights as co-efficients, a discriminant equation is constructed. The score is obtained by substituting the values in the following equations.

$$Y = 0.253\ X1 + 0.222\ X2 - 0.384\ X3 + 0.766\ X4 + 0.412\ X5 + X6 + 0.546\ X7 + 0.380\ X8 - 0.136\ X9 - 0.870$$

A constant term -0.87 was added merely to avoid negative value of score. The mean score for relapse group were computed as Y(R) = 4.2735 and Y(NR) = 2.7046 respectively.

Now a decision rule is to be established i.e. to determine the value of discriminant / critical score so that one can predict relapse. In the present study it is calculated by simply taking arithmatic mean of two scores Y(R) and Y(NR). The critical score thus worked out is 3.489. If the value of the score for a given patient calculated from above discriminant equation is more than the critical value, then relapse and if less then, nonrelapse of disease is predicted.

Observation

At this point an attempt is made to see how well this model will work in practice by using check sample of 24 patients whose data was not used for construction of discriminant function and establishing critical score. Thus this sample may be considerd as independent reasonable fascimile of future group of patients completing the treatment. The score was calculated for these patients and prediction of relapse was made for each patient. Table V shows the extent to which the predictions are true. From these results it will be seen that out of 12 patients belonging to nonrelapse group, 11 could have been predicted so on the basis of information available at the time of declaring these patients as cured. Similarly 11 patients out of 12 relapsed group could have been predicted of unfavourable forecast. Thus the sensitivity of model is 91.67% and specificity is 91.71%. It indicates that in 92% of total cases of tuberculosis, this model can predict a correct outcome (Accuracy of the model).

However, for a physician, whenever an individual patient is under consideration, one is interested in knowing the predictivity

of present scoring system i.e. how correctly the given results predict the outcome of the treatment given the score results. In other words what is the probability of truly predicting the relapse when score is more than critical score or probability of non-relapse when patient score is less than critical score. This can be estimated by calculating predictive value of score.

As the predictive value is affected by prevalance of disease in population; It was estimated on hypothetical population having relapse rate of 4%. The distribution of population in various categories according to score is shown in table VI.

The predictive value of positive score, i.e. score more than 3.489 works out to be 31.44% while for negative prediction i.e. score less than critical value, predictive value is 99.6%. This indicates that when the score of patient is more than the critical score, there are 31.44% chances that patient will have relapse within 3 years. On the other hand if the estimated score for a particular patient is less than critical score, then the probability of nonrelapse is 99.61%.

This information can be used for deciding the follow up strategy for the patient. If patient has critical score less than 3.489 than the chances of the relapse are minimal and hence follow up is not required. On the other hand if the score is more than critical score then the chances of relapse are 31% and hence patient should be kept under regular follow up. From the table it will be seen that majority of patients i.e. 883 who have score less than critical score need not be followed while 116 patients who have score above the critical score only need to be followed for relapse.

This reduces the burden of health services tremendously. This also shows that negative predictive value can be of greater utility as it more accurately predicts the outcome.

Discussion

In the present study an attempt is made to develop and propose a quantitative rationale to categorise the patients in "relapse" or "nonrelapse" group and decide on post treatment managemant of the disease. Thus the decision on follow up strategy can be based on more sound formulation. There is scope for further improvement in this model and the obvious is to look for other more

significant predictor. One such predictor could be initial colony count at the time of detection. This could not be included in present model as the information was not available. The presence of HIV infection is very important as this problem is now emerging. Other factors could be occupation of patient, concurrent illness etc. Another limiting factor is period of follow up which is 3 years from the date of cure. A longer follow up may yield different results.

Apart from these on mathematical side the model can be further improved. The cut off point for critical score is obtained just by arithmetic means. However ROC at different cut off points can be calculated and most accurate value of score can be estimated.

Despite these limitations, the author believes that this study has merit of statistical prediction and decision making for better follow up.

Table I

Table showing Numerical Coding of each variable.

Variable	Description	Numerical coding	
X1	Sex	Male	= 1
		Female	= 2
X2	Stage of disease on diagnosis	Minimal	= 1
		Mod. advance	= 2
		Far advance	= 3
X3	Side of lesion	Unilateral	= 1
		Bilateral	= 2
X4	Cavitation on diagnosis	Absent	= 1
		Present	= 2
X5	Residual Cavity	Absent	= 1
		Present	= 2
X6	Period of sputum conversion	< 15 days	= 1
		> 15 days	= 2
X7	II defaulter action required	Not required	= 1
		Required	= 2
X8	Drug regimen given	2SHRZ/6 TH	= 1
		2HRZ/6 TH	= 2
		2SHT/16TH	= 3
X9	Weight difference	Increased	= 1
		Decreased	= 2
		No Change	= 3
X10	Criterion	Non-relapsed	= 1
		Relapsed	= 2

Table II

Means of nine variables in relapse and nonrelapse and on entire normative sample with Standard Deviation.

Variable	Relapse Group	Nonrelapse Group	Normative Sample	
			Mean	S.D.
X1	1.40	1.30	1.350	3.020
X2	1.95	2.40	2.175	5.070
X3	1.45	1.40	1.425	3.710
X4	1.15	1.70	1.425	3.710
X5	1.05	1.35	1.200	3.400
X6	1.00	1.55	1.275	2.820
X7	1.20	1.70	1.450	3.140
X8	1.40	1.70	1.550	3.140
X9	1.15	1.20	1.750	3.570

Table III

Correlation matrix for predictor and criterion (group membership) variable.

Variable	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10
X1	1.000									
X2	-.029	1.000								
X3	-.085	0.532	1.000							
X4	-.174	0.320	0.129	1.000						
X5	-.175	0.151	0.127	0.127	1.000					
X6	-.217	0.145	-.065	0.031	-.021	1.000				
X7	.074	-.197	.116	-.141	-.150	0.344	1.000			
X8	-.179	0.135	.287	-.030	-.131	0.107	0.111	1.000		
X9	.051	0.153	.153	.304	.132	0.206	0.253	0.191	1.000	
X10	-.105	0.280	-.128	.469	.186	0.617	0.302	0.301	0.310	1.000

Table IV

Table showing Beta -weights , Bi - weights and Discriminant weights of different Variables.

Variables	Beta - Weight	Bi-weight	Discriminant Weight (Vi - Weight)
X1	0.131499	0.137682	0.253210
X2	0.194045	0.120855	0.222264
X3	-.244740	-.208590	-.383610
X4	0.488843	0.416636	0.766231
X5	0.241132	0.224253	0.412422
X6	0.484937	0.543748	1.000001
X7	0.295417	0.296919	0.546061
X8	0.205584	0.206630	0.380011
X9	-.083290	-.073690	-.135520

Table V

Outcome of prediction of Relapse and Nonrelapse on a Check Sample.

Critical Score	Prediction By Equation	Actual Status		Total
		Relapse	Non-relapse	
>3.489	Will relapse	11	1	12
<3.489	Will not relapse	1	11	12
	Total	12	12	24

$$\text{Sensitivity} = \frac{\text{True +ves}}{\text{True +ves + False -ves}} \times 100 \qquad \text{Specificity} = \frac{\text{True -ves}}{\text{True -ves + False +ves}} \times 100$$

$$= \frac{11}{11+1} \times 100 \qquad = \frac{11}{11+1} \times 100$$

$$= 91.67 \qquad = 91.67$$

Table VI

Predictivity of present scoring system with relapse rate of 4% (Hypothetical Population)

Critical Score	Relapse	Non Relapse	Total
>3.489	36.67	79.97	116.64
<3.489	3.33	880.03	883.36
Total	40.00	960.000	1000.00

Positive predictive value = 31.44%

Negative Predictive Value = 99.62%

References

1.Sarah, J.et al. Followup of patient with pulmonary tuberculosis adequately treated by Chemotherapy - Is this really necessary ! 1974 : Lancet 64 (2) 7881 : 641

2. Tsutomu Oyama and Maurice Tatsuoka Prediction of relapse in pulmonary tuberculosis. American review of respiratory diseases, 1956; 73 : 472 - 483 .

3. Fisher R. A. The use of multiple measurements in taxonomic problems Annual Euganics, 1936; 7:179 .

4. Agarwal, S.R. et al . Intermittent chemotherapy in treated cases of pulmonary tuberculosis. Indian journal of tuberculosis 1985; 31(3): 179 - 181.

5.William C. Balley. Evaluating the need for periodic recall and re-examination of patient with inactive pulmonary tuberculosis. American review of respiratory diseases 1973; 107 : 854 - 857 .

Typing Of Mycobacterium Tuberculosis With Special Reference To Biotyping

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Tuberbulosis remains a major public health problem world wide. Despite the implementation of efficient multidrug therapy and BCG vaccine, the incidence of this disease has changed little in developing countries. This indicates that the parameters of disease transmission have yet to be sufficiently understood to imporve global tuberculosis control.

Tuberculosis has represented a balance of interactions between susceptible host and the organism. Most probably the infecting organism in ancient times was M.bovis and the M.tuberculosis may be a variant of M.bovis adapted to human beings. During this adaptational phase, the tubercle bacilli acquired some phenotypic characters with the major difference in virulence for man and cattle[1]. The species M.tuberculosis for many years included both human and bovine tubercle bacilli. Another species associated with tuberculosis in Africans was named as M.africanum. However, because of their high DNA-DNA relatedness this species differentiation was questioned[2]. Hence, Grange[3] preferred to describe these variants as M.tuberculosis with a type designation and Tsukamura [4] refers to them as the 'M.tuberculosis complex' which includes M.tuberculosis, M.bovis, M.bovis (BCG),M.microti and M.africanum.

Though these variants belong to a complex, they differ in several important aspects from one-another. Dhaygude and Shah[5] first described a variant of human type of M.tuberculosis differing in its virulence in guinea pig. Grange et al [6] further studied them and noted that it was a distinctive phage type. It accounted for many cases of tuberculosis among Asian immigrants in London and it was

then named as Asian human variant of M.tuberculosis. Marks[7] recognised two variants of the bovine type, European and Afro-Asian. Yates and Collins[8] used four simple biochemical and cultural tests to distinguish two variants each of human and bovine type and this work was extended by Collins et al[2]. They used four tests such as oxygen requirement, nitrate reductase activity, susceptibility to pyrazinamide (60µg/ml) and susceptibility to thiophen-2- carboxylic acid hydrazide (TCH or T2 H) (5 ug/ml) to divide M.tuberculosis into five variants as Classical human, Asian human, Classical bovine, African I and African II. The variants studied were well correlated with different ethnic group of patients. According to them this subdivision has been shown to be useful in epidemiological studies of tuberculosis. Hoffner et al[9] while studying the biovars of M.tuberculosis replaced O_2 requirement test by niacin test.

The classical M.tuberculosis and its Asian variant are aerophilic, nitrate reductase positive and exhibit similar degree of virulence for man and cattle. The susceptibility to TCH differentiates them as Classical human being resistant to TCH and Asian human being sensitive. M.africanum is microaerophilic, nitrate reductase variable, less virulent to man and with doubtful pathogenicity in cattle. Classical M.bovis is microaerophilic, nitrate reductase negative, resistant to pyrazinamide and highly virulent for cattle. All variants except Classical human type are sensitive to 5 ug/ml of TCH. M.bovis (BCG) strains are aerophilic, nitrate reductase negative and not pathogenic for man and cattle [1,2].

Kothadia et al [10] followed the same biochemical tests as Collins et al [2] to subdivide 121 strains of M.tuberculosis from pulmonary tuberculosis cases into different biovars. Their study highlighted the limitations of this method as they observed that 93.4% strains revealed TCH resistance and hence were segregated as classical human type only. Similar finding (TCH resistance) was also reported by Mayurnath et al[11] and Goode D[12] regarding the M.tuberculosis strains from North India and Nepal respectively. It is generally believed that the South Indian strains of M.tuberculosis are TCH susceptible and are attenuated in guineapigs[13]. These strains are labelled 'Asian' type. Further it was attempted to study whether the difference in guinea pig virulence is reflected in difference in

virulence in man. However, by using the criteria as duration of symptoms, the radiological extent of the lesion and response to antituberculosis drugs, it was observed that there was no appreciable difference in human disease caused by the guineapig virulent (TCH resistant) and attenuated strains (TCH sensitive) [10-14]. The attenuated strains of M.tuberculosis (Asian type) do not give uniform results with conventional phages used for phage typing of mycobacteria and are referred as Intermediate (I) type. These strains are highly susceptible to hydrogen peroxide and TCH and they possess an attenuation indicator (AI) lipid in their cell envelope [15]. It is proposed that such strains form a significant subgroup of M.tuberculosis.

Phage typing of Mycobacteria, though appeared to be promising in initial phases, has subsequently failed to subdivide tubercle bacilli effectively. Depending on the sensitivity to Mycobacterial Typing Phage, Human (MPTH), e.g. 1, 2,3,4,5, and few auxiliary phages M.tuberculosis strains can be divided into Ao, Aox, B & C types. The available evidence suggests that phage type of mycobacteria is a constant feature of a given strain and does not readily change either in the host, on subculture or on mutation to drug resistance [16].

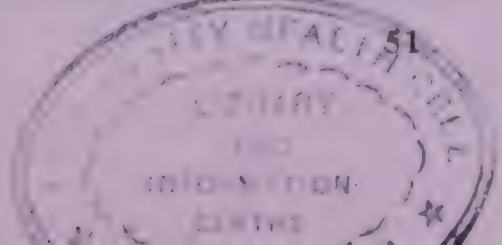
Because of certain limitations in subdividing M.tuberculosis by the techniques yet available, strain differentiation by DNA techniques has been recently developed. Since the discovery of polymorphic DNA in M.tuberculosis, strain differentiation has become an important tool in the epidemiology of tuberculosis for studying outbreaks, nosocomial infections, human immunodeficiency virus-associated transmission, and the dissemination of multidrug-resistant strains [17,18].

Among the genetic elements contributing to DNA polymorphism in M.tuberculosis, the insertion element IS6110 has been studied most intensively [19,20]. This 1,355 bp insertion sequence (IS) element is related to the IS 3 family of ISs and has been found exclusively in all isolates of the M.tuberculosis complex group of mycobacteria. Most M.tuberculosis complex strains carry multiple IS 6110 copies. However, unexpectedly, van Soolingen et al [17] encountered an Indian strain of M.tuberculosis which lacked IS 6110 DNA sequence. This observation offered some limitations to the use of IS 6110 DNA as a target for amplification by PCR for detection of M.tuberculosis in

clinical specimens. The authors also described large number of Indian strains with only single IS 6110 copy and hence they were difficult for differentiation by IS 6110 DNA fingerprinting, because this insertion segment was found to be associated with a unique restriction fragment in those strains. Therefore, in addition to IS 6110 four other targets for restriction fragment length polymorphism (RFLP) analysis have been described, (i) IS 1081 [21] (ii) a polymorphic GC-rich repetitive sequence (PGRS)[22] (iii) the major polymorphic tandem repeat (MPTR) sequence [23] and (iv) the 36-bp direct repeat (DR) sequence [24]. van Soolingen et al [17] have attempted to compare the RFLPs of each of these genetic markers in M.tuberculosis, especially strains containing few IS 6110 copies and to determine their suitability for strain differentiation.

The IS 1081 element was found to be less useful for strain differentiation because most of the copies of this element are present at identical positions in M.tuberculosis chromosome [17,25]. Similarly, MPTR sequence allowed only limited strain differntiation [17]. It could not segregate M.tuberculosis and M.bovis as strains of both species were found to belong to both sequavars LONG & MED-G[26]. It is postulated that the transition between M.tuberculosis and M.bovis has occured more than once during the adaptational phase of M.bovis for many generations in human beings. Many M.bovis isolates from humans have high IS 6110 copy numbers suggesting that these strains may be in transition from M.bovis to M.tuberculosis. This reemphasises the fact that there is a continumm of phenotypes in human isolates ranging from classical bovine to classical human type of M.tuberculosis with intermediate phenotypes [2, 9]. M.africanum belonged to sequavar SHORT and M.bovis (BCG) to MED-C[26].

The sequavar studies were carried out by using polymerase chain reaction restriction enzyme analysis (PCR-REA) and was found to be rapid, sensitive and specific especially for identification of important vaccine strain of M.bovis (BCG). Recently IS 1081 finger printing high performance liquid chromatography (HPLC) profiles of mycolic acid esters have been used satisfactorily to identify M.bovis BCG from M.tuberculosis and M.bovis strains. BCG is widely used as an immunotherapeutic agent in tumours in cancer patients, & hence it has become more important to distinguish it, reliably from virulent



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strains of the 'M.tuberculosis complex'.

According to van Soolingen et al [17, 18] repetitive DNA elements like the DR,MPTR & PGRS probes might be more useful than IS elements for measuring quantitative evolutionary relationships, because the DNA rearragements caused by these elements are likely to be more random in nature. And hence they can be used as differential molecular clocks to determine the evolutionary distances between M.tuberculosis strains.

The DR sequence and PGRS RFLP patterns have enough stability and various outbreak strains can be well recognised by using these newer sequences [18]. Different RFLP patterns of M.tuberculosis complex have been observed among strains originating from different geographical areas and various tuberculosis outbreaks. Identical RFLP patterns have been observed among paired strains from same patients, isolated from same sites or during relapse of the disease. This helps in understanding the pathogenetic mechanism of the disease. However, small variations do occur in RFLP pattern during strain cultivation. It is also observed that there is no correlation between the RFLP types and the susceptibilities of M.tuberculosis strains to antituberculosis drugs [28].

van Soolingen reported that RFLP patterns using DR sequences among Indian strains of M.tuberculosis are less polymorphic compared with those among the strains from other countries, suggesting that the Indian strains belong to an evolutionary related group of strains which is distinct from strains isolated on other continents.

Bovine tuberculosis is still a major infectious disease among cattle and other domesticated animals leading to great economical losses in agriculture. To study the epidemiology of bovine tuberculosis and to control it, strain differentiation of M.bovis by DNA techniques is widely applied. It has been observed that M.bovis from cattle, mostly contain single IS 6110 copy while, multiple IS 6110 copies were found in M.bovis strains isolated from animals other than cattle in zoos and wild parks. Such M.bovis strains with multiple IS 6110 copies were found in few Dutch patients indicating their source of infection. Hence IS 6110 marker can be used for typing of M.bovis depending on the origin of the strains. However, PGRS and DR associated RFLPs proved to be better tools for M.bovis typing [18].

Different typing methods of M.tuberculosis complex strains have become necessary in order to meaningfully correlate the bacterial isolations from different clinical forms of tuberculosis, drug resistant tuberculosis, nosocomial outbreaks of tuberculosis, HIV associated tuberculosis and from ethnic groups in different geographical areas of the world. These methods range from simple typing techniques such as morphotyping, cultural typing, biotyping to most advanced systems as DNA fingerprinting. However, quite often they are proved to be more or less inadequate or nondiscriminative for the purpose sought.

Nevertheless, typing of M.tuberculosis complex has become an urgent need of today in view of the changing trends in tuberculosis disease pattern. The simple procedures such as biotyping can be performed in moderately equipped laboratories with great benefits. We can estimate the prevalance of a particular biotype in a given clinical and epidemiological set up.

In our study [10], we extended our work and observed that most of the classical human strains of M.tuberculosis revealed 20ug/ml to 80ug/ml of TCH as minimum inhibitory concentration and there was a cross resistance between TCH and INH. It is speculated that this aspect may also be interesting for further discrimination among the strains.

It can be recommended that all the isolates of M.tuberculosis should be subjected to biotyping and then sent to reference laboratories for genetic studies. It is rather more relevant in Indian set up of tuberculosis disease (Indian strains of M.tuberculosis harbour low copy IS 6110 element or it may be lacking) to study the evolutionary pattern of M.tuberculosis strains in comparison with the strains from other countries and is more logical to know what factors might be responsible for such a change in their behavior.

References

1) Bates J.H.& Stead W.W. The history of tuberculosis as a global epidemic. Med.Clin N.Am. 1993; 77: 1205-1217.

2) Collins C.H. Yates M.D., Grange J.M.Subdivision of M.tuberculosis into five variants for epidemiological purposes methods and nomenclature. J.HYG. Camb. 1982; 89: 235-242.

3) Grange J.M The changing tubercle Br.Jr.Hos. Med.1979; 22: 540-548.
4) Tsukamura M.Numberical classification of slowly growing mycobacteria Inter. Jr.of Syste. Bact. 1976; 26: 409-420.
5)Dhaygude R.G & Shah B.R Variation in virulence of M.tuberculosis and its correlation with clinical type of tubercular disease. Ind.Jr.Med. Res. 1948; 36: 79-89.
6) Grange J.M., Aber V.R., Allen B.W., Mitchison D.A. and Goren M.B.The correlation of bacteriophage types of M.tuberculosis with guineapig virulence and invitro indicators of virulence Jr.Gen.Micobiol. 1978; 108: 1-7
7) Marks J.A. System for the examination of tubercle bacilli and other mycobacteria. Tubercle 1976; 57: 207-225.
8) Yates M.D.and Collins C.H.Identification of tubercle bacilli. Annales de Microbiologie. 1979; 130B: 13-19.
9) Hoffner S.E., Svenson S.B., Norberg R., Dias F., Ghebremichel and Kallenius G.Biochemical heterogeneity of M.tuberculosis complex isolates in Guinea/Bassau. J.Clin.Microbiol.1993;31: 2215-2217.
10) Kothadia S., Kale S., Powar R.M., Bannur M.,Jahagirdar V.L. and Saoji A.M. Subdivision of M.tuberculosis ioslated from pulmonary tuberculosis cases in and around Solapur (South India) into five variants. Ind., J.Tub. 1991;38: 213-216.
11) Mayurnath S., Ananthraman D.S., Bailey G.V.J., Radhamani M.P., Valishye R.S., Venkatraman P. and Tripathi S.P. Tuberculosis prevalence survey in Kashmir Valley. Ind.Jr.Med. Res. 1984; 80:129-132.
12) Goode D.Bacteriophage typing of strains of M.tuberculosis from Nepal. Tubercle. 1983; 64: 15-19.
13) Venkatraman P., Paramasivan C.N. Ilampuranam K.and Prabhakar R.Intraspecies differentiation of strains of M.tuberculosis obtained from Cze choslovakian, Mongolian and South Indian patients. Ind. Jr.Med. Res. 1988; 88: 211.
14)Grange J.M., Collins C.H.and Yates M.D. The 'Asian' type of M.tuberculosis. Ind. Jr.Chest Dis. & Allied Sc. 1982; 24: 88-93.
15) Goren M.B., Brokl O, Schafer W.B.Lipids of putative relavance to virulence in M.tuberculosis: phthiocerol dimycoserate and the attenuation indicator lipid, Infect Immun. 1974b, 9: 150-158.
16) Crawford J.and Bates J.H.Phage typing of mycobacteria. In : The Mycobacteria. a source book, Part A.Marcel Dekkar, Inc. Newyork, 1984; 123-132.
17) Dick van Soolingen, Petra E.W. DeHass, P.W.M.Harmans, Peter M.A., Groenen and Jan D.A. Van Embden. Comparison of various repetitive DNA elements as genetic markers for strain differentiation and epidemiology of M.tuberculosis. J.Clin.Microbiol. 1993;31:1987-1995.
18) Dick van Soolingen, Petra E.W. De Hass, Haagsma J.,Eger T.,P.WM.Hermans, Ritacco V., Alito A and Jan D A. Van Embder. Use of various markers in differentiation of M.bovis strains from animals and humans and for studying epidemiology of bovine tuberculosis. J Clin Microbiol. 1994; 32: 2425-2433.
19) Mc Adam R.A., P.W.M Hermans, D.van Soolingen, Z.F. Zainuddin, D.Catty, J.D.A. van Embden and J.W.Dale. Characterisation of a M.tuberculosis insertion sequence belonging to the IS 3 family Mol. Microbiol. 1990; 4: 1607-1613.
20) Thierry D., A.Brisson-Noel, V.Vincent-Levy-Frebault, S.Nguyen, J.Guesdon and B Gicquel Characterisation of a M tuberculosis insertion squence IS 6110 and its application in diagnosis J.Clin. Microbiol. 1990; 28: 2668-2673.
21) Collins D.M.and Stephen D.M.Identification of insertion sequence IS 1081 in M.bovis FEMS Lett 1991, 83 11-16
22) Ross C., Raios K.Jackson and Dwyer B.Molecular cloning of a highly repeated element

from M.tuberculosis and its use as an epidemiological tool. J.Clin Microbiol. 1992, 30 942-946.
23) Herman P.W.M., D van Soolingen, and J.D.A. van Embden Characterisation of a major polymorphic tandem repeat in M.tuberculosis and its potential use in epidemiology of M.Kansasii and M.gordonae. J.Bacteriol. 1992;174: 4157-4165.
24) Hermans P.W.M., D.van Soolingen, J.W Dale, E.M.Bik, P.E.W. de Haas, and J.D.A. van Embden. The insertion element IS 987 from M.bovis BCG is located in a hot-spot interation region for insertion elements in M.tuberculosis complex strains Infect. Immun. 1991; 59: 2695-2705.
25) D.van Soolingen, P.W.M.Hermans, P.E.W. de Haas and J.D A. van Embden. Insertion element IS 1081-associated restriction fragment length polymorphism in M.tuberculosis complex species; a reliable tool for recognising M.bovis BCG J.Clin. Microbiol. 1992; 30: 1772-1777.
26) Frothingham R.Differentiation of strains in M.tuberculosis complex by DNA sequence polymorphisms, including rapid identification of M.bovis BCG J.Clin. Microbiol. 1995;33: 840-844.
27) Floyd M.M., Silcox V.A., Jones W.D.Jr., Butler W.R.and Kilburn J.O.Separation of M.bovis BCG from M.tuberculosis and M.bovis by using higher performance liquid chromatography of mycolic acids. J.Clin. Microbiol 1992;30: 1327-1330.
28) Dellagi D.C., Abderrahman A., Haltiti R., Koubagi H., Gicquel B.and Dellagi K.Large scale DNA fingerprinting of M.tuberculosis strains as a tool for epidemiological studies of tuberculosis. J.Clin. Microbiol 1993; 31: 2446-2450.

Immunodiagnostic Studies Using Mycobacterium Tuberculosis H 37Ra Excretory-Secretory Antigen In Pulmonary And Extra-Pulmonary Tuberculosis

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ABSTRACT

Tuberculosis is a major public health problem with about 1700 million people infected worldwide. In India alone , 12-13 million people are infected with M. tuberculosis. ELISA with penicillinase as marker has been developed using purified M.tuberculosis excretory-secretory (M.tb ES) antigen for detecting IgG antibodies by indirect ELISA and antigen by sandwitch ELISA. In pulmonary tuberculosis Indirect ELISA for antibody detection using EST-6 antigen showed a sensitivity of 84% and specificity of 91%, whereas sandwitch ELISA for antibody detection using affinity purified antibodies showed a sensitivity of 81% and specificity of 79%. In extra-pulmonary tuberculosis group, tuberculous antibodies to EST-DE1 antigen could be detected in 83% of osteoarticular tuberculosis and 80% of abdominal tuberculosis, while tubercular antigen positivity was seen in 69% of osteoarticular tuberculosis and 87% of abdominal tuberculosis. The combination of both the assays for analysis of samples gave a sensitivity of 73% and specificity of 98% in pulmonary tuberculosis and a sensitivity of 74% and a specificity of 96% in extra-pulmonary tuberculosis.

Introduction

Tuberculosis continues to be a major public health problem in many parts of world. The incidence of pulmonary tuberculosis is more frequent than extra-pulmonary form of the disease[1] Presently available methods of diagnosing the tuberculosis, such as smear examination and sputum culture to detect the presence of AFB, tuberculin skin test and radiological examination are either insensitive or time consuming. Hence, there is an urgent need of simple and reliable method for precise diagnosis of tuberculosis for initiating prompt treatment and containment of the disease.

In view of these limitations, continuing efforts have been made to develop suitable serological tools. In the past four decades, several immunological methods have been explored in diagnosis of tuberculosis. Methods such as gel diffusion, immunoelectrophoresis, and latex agglutination tests have been replaced by more sensitive techniques like radioimmuno assay (RIA) and enzyme linked immunosorbent assay (ELISA)in recent years[2,3].

Several serological assays with various modalities have been developed and explored for antibody, antigen and immune complex detection. However, most of the assays reported were either insensitive or lack the required specificity[4,5] for the reason that most of antigenic moieties and polyclonal antibodies used in such assays where common to many bacterial species, leading to cross reactivity and exihibited low diagnostic potential in endemic areas due to environmental mycobacterial exposures.

Over the years various mycobacterial antigens ranging from crude (culture filtrate, whole cell sonicate, PPD) to purified antigens (Antigen-5, glycolipid antigens, lipoarabinomannan) have been explored in the immunodiagnosis of tuberculosis. The groups of secreted antigens have drawn special attention of many workers for the fact that these antigens are believed to be more or less similar to the *in vitro* released mycobacterium antigens [6].

Earlier we have reported the diagnostic potential of M.tb ES antigen in the diagnosis of pulmonary and extra-pulmonary tuberculosis[7,8]. The present communication reports on the utility of partially purified M.tb ES antigens and polyclonal antibodies raised against them in detection of tuberculous antibody and antigen in pulmonary

and extra-pulmonary tuberculosis.

Detection of tuberculous IgG antibody and circulating tubercular antigen in pulmonary tuberculosis.

Mycobacterium tuberculosis H37Ra excretory-secretory (ES) antigen was prepared by *in vitro* culture of the bacilli in synthetic Souton medium as described earlier[7]. M.tb ES antigen was then partially purified by 6% trichloroacetic acid precipitation. The TCA supernatent fraction (M.tb EST antigen) was further fractionated in SDS-PAGE to obtain an active M.tb EST-6 antigen with molecular mass range of 37-45 KDa. M.tb EST-6 antigen was used in Stick Indirect ELISA to detect tuberculous IgG antibodies.

For the detection of circulating tuberculous antigen, Sandwich ELISA was developed using affinity purified goat anti EST antigen antibodies as described earlier[9].

Table 1

Detection of Tuberculous IgG Antibody and Circulating Tubercular Antigen in Pulmonary Tuberculosis.

Group	Total Number screened	No. showing positivity*		
		Antibody by indirect ELISA	Antigen by sandwich ELISA	Dual antibody & antigen
A.Pulmonary TB (smear positive)	58	49(84%)	48(83%)	44(76%)
B.Pulmonary TB (smear negative)	05	04(80%)	03(60%)	02(40%)
C.Disease control	28	03(11%)	05(18%)	00 (-)
D.Healthy control	29	02(7%)	07(24%)	01(3%)

* Sera samples showing tuberculosis IgG antibody titre of > 600 and tubercular antigen titre of > 100 were considered positive.

The results of analysis of 120 sera for tuberculous IgG antibodies and circulating tubercular antigen are summerized in Table 1. While the antibodies were detected with a sensitivity and specificity of 94% and 91%, the antigen assays showed 81% sensitivity and 79% specificity. Presence of both the antibodies and antigen was observed in 46 out of 63 tuberculosis sera, in none of the 28 disease control and

only one out of 29 healthy controls. Accordingly using a combination of both the assays for antibody and antigen it was possible to detect pulmonary tuberculosis cases with a sensitivity of 73% and specificity of 98%.

Detection of tubercular IgG antibody and circulating tubercular antigen in extra-pulmonary tuberculosis.

M.tb EST antigen was purified on DEAE ion exchange column chromatograpy to obtain an active antigen fraction EST-DE1 (unbound fraction). EST-DE1 antigen gave fairly comparable results to that of M.tb EST-6 antigen in diagnosis of pulmonary tuberculosis and further it was observed that it contained two of the active SDS-PAGE fractions of M.tb EST antigen i.e. M.tb EST-4 & 6 (Lodam A.N. and Harinath B.C., unpublished observations).

Stick Indirect ELISA using EST-DE1 antigen and Sandwich ELISA using affinity purified goat anti EST antigen antibodies were used for detection of tuberculous IgG antibodies and antigen respectively in extra-pulmonary tuberculosis cases.

In osteoarticular tubreculosis antibody detection showed a sensitivity of 83% and specificity of 80%, whereas antigen detection assay showed a sensitivity of 69% and specificity of 91%. In abdominal tuberculosis the sensitivity of antibody and antigen assays were 80% and 87% and specificities were 90% and 97% respectively.

Antigen detection assays was found to be better than antibody assay in extra-pulmonary tuberculosis, whereas combination of both the assays showed increased specificities in osteoarticular tuberculosis (95%) and abdominal tuberculosis (97%).

Using culture filtrate antigen specificity of 95-100% has been reported though the sensitivity was relatively low and varying[10,11]. Even purified antigens offer only marginal if any advantage in the diagnosis of tuberculosis [12,13,14]. For detecting mycobacterial antigens in body fluids, imuno assays with variable results have been reported. Batchuin et.al.[15] reported a sensitivity of 87% and specificity of 93% in detection of tubercular antigen in circulation. Though the reported results of individual systems for either antigen or antibody are encouraging, the improvement of serological diagnosis may be

obtained by simultaneous detection of antibody and antigen. Increased sensitivity was observed by Radhakrishnan et al.[12] and Krombovitis et al .[16], when the combination of the results from an antigen capture immunoassay and antibody measuring assay were offered to improve diagnosis of tuberculosis over a single antibody test. In the present study we do not observe any improvement in the sensitivity by the combination of both the assay systems in the diagnosis of tuberculosis. However, improved specificity was obtained by the combination of both the assay systems. **Thus it can be stated that combined antibody and antigen assays will be better than any of the single assay system for precise diagnosis of pulmonary and extra-pulmonary tuberculosis for judicial use of anti-tubercular therapy.**

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References :

1. Sudre P, ten Dan G, Kochi A. Tuberculosis a global overview of the situation today. Bull. WHO, 1992; 70(2):149-159.

2 Kardjito TG, Grange JM, Beek JS. Cooperative studies on immunology of tuberculosis at Airlanga University Surbya, Indonesia. Asian Pacific J. Aller. and Immunol. 1986; 8: 141-146.

3. Yenez MA, Coppola MP, Russo DA, et al. Determination of mycobacterial antigens in sputum by enzyme immunoassay. J. Clin. Microbiol. 1986; 23:822-825.

4 Takahashi Y Specific serum agglutination of Kaolin particles sensitized with tubercle phosphate and clinical evolution as a serodiagnostic test for tuberculosis. Am. Rev. Respir. Dis. 1962; 85:709-719.

5. Chandrashekharan S, Venkatraman, Gupta EV, Chouhan MM et al Serodiagnosis of pulmonary tuberculosis by Kaolin agglutination test. Ind. J. Tub. 1990; 37. 11-14.

6 Grange JM The rapid diagnosis of paucibacillary tuberculosis Tubercle 1989;70 1-4.

7 Bhaskar A, Pradhan P, Chaturvedi P, Basak A, Lodam A, Narang P, Harinath BC Immunodiagnosis of childhood pulmonary and extra-pulmonary tuberculosis using Mycobacterium tuberculosis ES antigen by penicillinase ELISA. Annal. Trop Paediatr. 1994,14.25-30

8. Lodam AN, Pramanik J, Reddy MVR, Narang P, Kalantri SP, Gupta OP, Harinath BC. Diagnostic use of purified M.tb. EST-6 antigen in antibody detection and affinity purified anti M.tb EST antigen antibody in antigen detection in pulmonary tuberculosis. J. Immnoassay (in communication).

9. Lodam AN, Reddy MVR, Narang P, Gupta OP, Harinath BC. Fractionation, analysis and diagnostic utility of Mycobacterium tuberculosis H37Ra excretory-secretory antigen in pulmonary tuberculosis. Ind. J. Biochem. Biophy. 1996; 33:66-71.

10. Nassau E, Parson ER, Johnson GD. The detection of antibodies to Mycobacterium tuberculosis by enzyme linked immunosorbent assay(ELISA). Tubercle. 1976;57:67-70.

11. Benjamin RG, Danial TN. Serodiagnosis of tuberculosis using the enzyme linked immunosorbant assay (ELISA) of antibody to Mycobacterium tuberculosis antigen 5. Am. Rev. Respir. Dis.1982;126:1013-1016.

12. Radhakrishnan BV, Mathai A. ELISA to detect M.tuberculosis antigen 5 and anti mycobacterium antibody in cerebrospinal fluid of tuberculous meningitis. J. Clin. Lab. Anal. 1991; 5:233-237.

13. Sada DE, Ferguson LE, Danial TM. An ELISA for the serodiagnosis of tuberculosis using a 30,000 DA native antigen of Mycobacterium tuberculosis. J. Infect. Dis. 1990;162:928-931.

14. Sachan AS, Gupta RK, Gupta D et al. Diagnostic significance of antigen A-60 by ELISA test in pulmonary tuberculosis. Ind. J. Tub.1994; 41:239-243.

15. Batchuin N, Watgwajata S, Pumprueg V et al. Value of an ELISA for mycobacterial antigen detection as a routine diagnostic test of pulmonary tuberculosis. Asian Pacific J. Aller.Immunol 1990;8:5-11

16. Krombovitis E, Harris M, Hughes D.TD. Improved serodiagnosis of tuberculosis using two assay test. J.Clin. Pathol. 1986;39:779-785.

Characterisation Of *Mycobacterium Avium* Complex (MAC) Isolates Obtained From Clinical Samples And The Environment Of The South Indian BCG Trial Area

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In the large scale BCG trial in South India during 1968 to 1980, BCG did not offer any protection against the bacillary form of pulmonary tuberculosis (Tuberculosis prevention trial, 1980). The study population in this trial was characterised by a high prevalence of tuberculous infection and also by a very high prevalence of non-specific sensitivity. Thus , more than 90% among those aged 14 years and above had positive skin-test reaction to PPD-B (Narain et al, 1978). Further, NTM were obtained from 6% of sputum samples of subjects in this area (in 1 year) and nearly 20% of them belonged to what was then termed the *M.avium-intracellula re-scrofulaceum* complex (Paramasivan *et al*, 1985). One of the several hypotheses put forward to explain the results of this trial is that prior exposure to NTM, present in the environment of this area, could have played a role in modulating the immune response to subsequent BCG vaccination. Systematic investigations on the isolation and identification of mycobacteria in the environment of this area and comparison of the NTM profile in the environment with that of sputum samples from subjects residing in the same area had

not been done earlier. Therefore, a study was undertaken by us in 1992 to isolate and identify the strains of NTM prevalent in the environment of this area and also to find out any seasonal variation in their occurrence. The environmental samples included soil, water and house-dust. NTM isolates obtained from sputum samples at the same time from subjects residing in the same area were also included for comparison. This study indicated that soil samples predominantly yielded isolates belonging to the *M.fortuitum* complex while water , dust and sputum samples yielded isolates belonging to the *M.avium* complex (MAC), irrespective of the season (Kamala et al, 1994), based on the results of conventional phenotypic tests.

Since organisms identified phenotypically as belonging to MAC were among the predominant isolates obtained from the environment of the South Indian BCG trial area and since it has been shown that MAC strains show considerable variation in their *in vitro* drug susceptibility, it was considered that antimicrobial susceptibility testing of MAC isolates could generate patterns which could be used for comparing between isolates from the environment and sputum. A total of 62 MAC isolates from soil, water, dust and sputum including 31 each non-pigmented and pigmented , were tested for their susceptibility patterns . The susceptibility patterns generated in this study were unable to distinguish between isolates from the environment and sputum (unpublished observations).

In this study , isolates from the different sources phenotypically identified as MAC were further characterised using plasmid profiles (n=60 ; at the Tuberculosis Research Centre [ICMR], Madras),identification by 3 specific DNA probes, namely, DT1, DT6(n=50 ; at the Pasteur Institute, Paris) and the LiPA (Line Probe Assay) species probe (n=50 ; at the Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium in collaboration with Innogenetics, NV , Ghent), analysis of lipids using gas chromatography-mass spectrometry (GC-MS) (n=41 ; at the Lund University, Sweden), and analysis of mycolic acid pattern using thin-layer chromatography (TLC) (n=40 ; at the Prince Leopold Institute of Tropical Medicine, Antwerp, Belgium).

Plasmid DNA analysis of a total of 62 isolates classified as MAC

according to their phenotypic characteristics including 13 each from water and dust, 16 from soil and 18 from sputum showed that the maximum number of plasmid-carrying strains were among the non-pigmented strains from water while strains from sputum yielded few plasmids . In earlier studies on organisms that had been classified as MAC , plasmids have been reported to be preferentially found in clinical and aerosol isolates.

This work was done by these different laboratories in a mutually exclusive manner on a limited number of strains from the South Indian BCG trial area which had been phenotypically identified as members of the *M.avium* complex (MAC) . Though the results indicate the heterogenecity of environmental mycobacteria, an overall clustering as MAC was observed regardless of the approach used. Based on these encouraging results, this work is being extended on more number of strains in order to further validate each of these approaches.

References

- Kamala, T., Paramasivan, C.N. , Daniel Herbert, Venkatesan, P., Prabhakar, R. Isolation and identification of environmental mycobacteria in the *Mycobacterium bovis* BCG trial area of South India. Appl.Environ.Microbiol. 1994 ; 60 : 2180-2183.
- Narain, R., Vallishayee, R.S., Reddy, A.V. Value of dual testing with PPD-B . Indian J.MedRes. 1978; 68: 204-219.
- Paramasivan, C.N., Govindan, D., Prabhakar, R., Somasundaram, P.R., Subbamal, S., Tripathy, S P. Species level identification of non-tuberculous mycobacteria from South Indian BCG trial area during 1981. Tubercle 1985 ; 66 :553 - 568.
- Tuberculosis Prevention Trial . Trial of BCG vaccines in South India for tuberculosis prevention Indian J.Med.Res. 1980 ; 72 (suppl.): 1-74.

Radiology Of Pulmonary Tuberculosis

P N Sharma

Tuberculosis is a cause for growing concern, and modern chemotherapy advances in case finding and holding notwithstanding, shows no signs of decline. It affects practically all organs of the body: lungs are favourite sanctuary site for tubercle bacilli.

Radiology plays a major role in detection of pulmonary tuberculosis. While chest X-ray continues to play a major role in detection of lung lesions caused by acid fast bacilli, it is important to note that a chest film is neither sensitive nor specific for the diagnosis of pulmonary tuberculosis; a negative chest X-ray doesn't rule out tuberculosis, nor would an abnormal film necessarily confirm the diagnosis. Add to this an inherent intra-observer and inter-observer error in interpreting lung lesions, a casual and careless interpretation of shadows on chest X-ray, short of clinical details and microbiological data, can lead to false- positive as well as false-negative diagnosis.

Radiologically speaking, pulmonary tuberculosis can be classified into following types: (a) primary (b) progressive primary (c) post primary. Primary lesions are characterised by a small peripheral area of consolidation called 'Ghon's Focus' where from the bacilli drain into the regional lymph nodes. The regional lymph nodes grow up and the pulmonary lesion and adenopathy is together called 'Ghon's Complex'.



Fig. 1 Ghon's Complex

The natural defence army of the body, particularly lymphocytes and macrophages, arrest the progression of lesion, which heals by fibrosis with or without calcification. These lesions are no longer monopoly of childhood tuberculosis, but have also started making their presence felt on adult skiagrams also. An inactive lesion may fail to cast any shadow on X-Ray or may present with (a) calcification: intrapulmonary or nodal (b) non-cavitating, subpleural consolidation or (c) a unilateral hilar, paratracheal or mediastinal adenopathy. The lymph nodes can grow sufficiently large and can compress a bronchus leading to obstructive atelectasis, erode a bronchus leading to dissemination of infection. Occasionally the damaged and eroded bronchi get permanantly dilated and distorted, a condition called bronchiectasis. The acidfast bacilli can travel to neighbouring structure and can cause miliary tuberculosis, pericarditis, pleuritis, haemotogenous spread of infection, vena cava compression and fistula formation. Rarely patients present with tuberculoma, a localised nodular parenchymal lesion which neither cavitates nor gets calcified.

Post primary tuberculosis is also called reactivation or secondary or adult tuberculosis. An adult, previously sensitised by acid fast bacilli either by infection or BCG vaccination, manifests by hypersensitive reaction to the tubercle proteins.

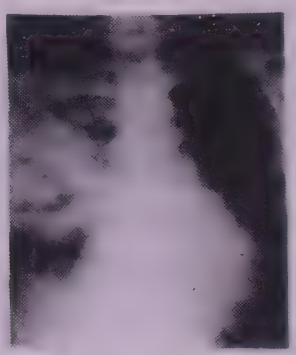


Fig. 2
TB lung abscess with fibrosis

There are several specific features of post-primary tuberculosis: (a) Site: Apico posterior segment of the upper lobe or superior segment of the lower lobe is commonly affected (b) lesion; a patchy and/or multifocal consolidation with nodular elements called 'acinar nodules' (c) Cavitation: Single or multiple, small or large, occasionally with air-fluid levels (d) fibrosis: upper zone nodular or linear opacities with features of marked volume loss.

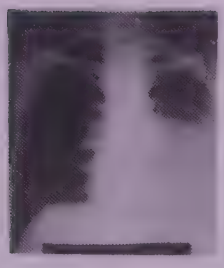


Fig 3. Left TB pleural effusion

The disease can spread by bronchogenic route and may be associated with / result in pleural effusions, miliary tuberculosis, tuberculoma. A secondary invasion of a tubercle cavity by aspergillus leads to characteristic fungal balls within the cavity. Similarly empyema, laryngeal disease, bronchial obstruction are well known complictions of tuberculosis.

Lesions simulating pulmonary tuberculosis

The lesions can be divided into :

(a) Artifacts

(b) Other diseases

(a) **Artifacts:** A hair braid, young breast shadow, thymus, skin folds etc can all give rise to shadows which mimic parenchymal lesions caused by tuberculosis.

(b) **Differential diagnosis of pulmonary tuberculosis.**

A number of diseases can masquarade as pulmonary tuberculosis, conversely pulmonary tuberculosis shares many of the radiological features of a wide range of respiratory diseases. An interpretation of chest X-ray in a suspected case of tuberculosis ought to take into consideration historical details, physical signs and microbiological profile of the patient. It must be differentiated from a wide range of other diseases including:

(1) Pneumonia- Staphylococcal and Klebsiella pneumonia, particularly unresolved and cavitating may mimic tuberculosis on radiography.



Fig. 4 Intrathoracic Sarcoidosis

(2) Pulmonary Fibrosis due to sarcoidosis, extrinsic allergic alveolitis, pneumoconiosis and silicosis often produce bilateral reticular, reticulonodular or nodular lesions with or without hilar adenopathy.
(3) Carcinoma Lung: Cavitating squamous cell carcinoma may be confused with postprimary tuberculosis.
(4) Allergic bronchopulmonary aspergillosis: a central bronchiectasis and intraparenchymal lesion can resemble tuberculosis.
(5) Lymphoma: Mediastinal adenopathy can be a presenting feature of tuberculosis as well as malignant lymphoma.

Tuberculosis and HIV

Tuberculosis is common among HIV infected people and can be the earliest sign of immunosuppression. Pulmonary manifestations of HIV associated tuberculosis differ in several ways from usual tuberculosis.
(a) The disease tends to present as reticular, reticulonodular wide spread bilateral lesions

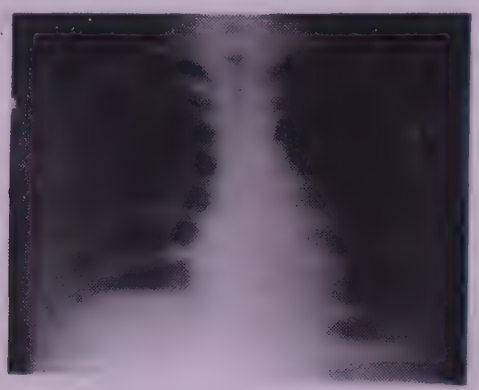


Fig. 5
Miliary TB in HIV

(2) Miliary pattern is more often found
(3) lower zones are as often picked up as upper ones
(4) The disease is less often cavitating and smear positive.

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Mycobacterial Chemotherapy And Prophylaxis In Immunocompromised Host

P Narang

Once an obscure terminology, "Immunocompromised Host" has of late acquired a very important status amongst the various syndromes. The condition is no longer limited to a passing reference in the text; on the contrary, books on this subject are now increasingly available. According to Paul Russel [1] a compromised host lies open, as a form of exposed, all purpose culture plate. He not only admits many kind of ambient organisms with ease, but usually does so in relative silence. Thus, the recognition of infection in them requires awareness and keen observation on the part of the physician. After diagnosis, proper strategies for treatment and further prevention need to be adopted, as these patients are even more compromised in their effort to get rid of the pathogen on their own.

A number of infective and non infective conditions could be listed as the causes of immunocompromised state of an individual (Table-1) but today the infection with Human Immunodeficiency virus has surpassed them all. The paradox is that this infection further prepares the ground for other agents to establish themselves. Mycobacterial infections (Table-1) particularly M.tuberculosis has surfaced as one of the most deadly combination in these patients. Persons who are coinfected with Mycobacterium tuberculosis and HIV develop active tuberculosis at a rate of approximately 8-10% per year compared to the estimated 5-10% life time tuberculosis risk in persons with intact immune system. Moreover, HIV infected individuals who acquire new infection with M.tuberculosis have a higher risk of developing tuberculosis very rapidly. When this

organism is introduced as primary infection in this population group the attack rates are as high as 37% to 39% [4] and within a brief period of 4-8 weeks infection progresses to active disease leading to high rates of morbidity and mortality. [5] The other non tuberculous mycobacteria (NTM)have also been increasingly isolated from immunocompromised patients particularly those with HIV infection. The epidemiology and pathogenesis of these are quite distinct from that of M.tuberculosis as these organisms primarily have environmental or animal reservoirs and person to person transmission has not been clearly demonstrated. Infection generally results from inhalation of contaminated dust or water or direct inoculation of contaminated material on the skin. Virulence of these organisms is also much less than that of M.tuberculosis and therefore they are more often than not associated with immunocompromised states- where they may cause disseminated disease. Mycobacterium avium and Mycobacterium intracellulare are closely related species grouped together as M.avium complex (MAC). These followed by M.kansasii have now been recognised as important isolates from HIV infected individuals [6].

Treatment Of Mycobacterial Infections

Rationale

A number of regimens for treatment of tuberculosis have been tested but the physician treating immuno-compromised patients must choose a regimen which is efficacious and less toxic. Multiple drug regimens should be used in the initial phase to prevent emergence of drug resistance and the Mycobacterial isolates must be subjected to antibiotic sensitivity testing so as to later modify the regimen accordingly. These subjects generally need prolonged therapy because of impaired host mechanism and uncertainty of standard regimens [7,8].

TB therapy is effective in HIV infected individuals when therapy is begun promptly. There are many reports to indicate that seropositive patients with TB respond just as well as seronegative patients [8,9,10]. However, when appropriate anti TB therapy is delayed either because of unsuspected drug resistance or the lack of diagnosis, mortality increases. Perriers et al[11] report a mortality of 15% in subjects where treatment was started within 15 days and 36% in those where it was initiated 23 to 119 days later.

Chemotherapy

I. WHO has categorised tuberculosis patients and given treatment regimens for each of the four categories [12]. Tables 2, 3 & 4 depict the advocated regimens for adults of >33kg weight in categories I, II and III. The regimens for HIV infected persons in these categories are the same except for the following modifications.

1) WHO recommends fully supervised treatment in initial phase for category 1 and II patients.(Table 2,3). It is recommended in all settings but should be adhered to particularly in countries with scarce resources and high incidence of HIV infection. For these are the countries where we need to prioritize patients and maintain an acceptable level of quality care. Also to reduce drug costs, such patients may receive daily supervised treatment in the first month of the initial phase, followed by administration of the same drugs 3 times weekly in the second month. Then a 6 months self administered continuation phase should be given.

2) Considering the risk associated with parentral therapy in HIV patients, streptomycin should be replaced by ethambutol.

3) Category III patients (Table 4) i.e. pulmonary smear negative tuberculosis with limited parenchymal involvement and extra pulmonary tuberculosis (other than the clinical forms considered in category 1) may be considered for self administration during the initial phase, if fixed dose combination tablets containing INH & rifampicin (with pyrazinamide also included or given separately) of proven bioavailbility are available.

4) Adverse hypersensitivity reactions in HIV positive patients have been observed with thioacetazone and hence this drug should be avoided and replaced by ethambutol.

5) Desensitization practices for any anti TB drug should be avoided because of serious toxicity and death in these patients

In countries where rifampicin and ethambutol are not available in sufficient quantities, both for individual and public health reasons, patients with tuberculosis must be treated with the most effective therapy available and in the absence of rifampicin and ethambutol, thiacetazone may be included, but then the patients should be warned about the possibility of skin reactions and advised to discontinue it at once if skin reactions occur.

II. The recommendations of CDC [16] and American Thoracic Society [15] for HIV positive and HIV negative subjects is shown in Table-5. The recommendations are based on susceptibility of organisms to antimycobacterials. The initial therapy in subjects with no suspicion of drug resistant organisms should be, isoniazid (H), rifampicin (R), pyrazinamide (Z) given for 2 months followed by isoniazid and rifampicin for 7 months or 6 months after the cultures are negative, whichever is longer.

In case primary drug resistance is suspected then the initial phase should consist of a fourth drug ethambutol in addition to the three drugs mentioned above; (for reasons already stated streptomycin is avoided in these patients) and in case INH resistance or intolerance occurs then treatment should be with rifampicin and ethambutol for 18 months or for 12 months after cultures are negative which ever is longer.

In case rifampicin resistance or intolerance is present isoniazid, pyrazinamide and ethambutol is recommended for 18 to 24 months or for 12 months after cultures are negative which ever is longer.

Multidrug Resistant Tuberculosis

An organism is said to be multidrug resistant if it is resistant to two or more drugs and about 50-100 million people worldwide are thought to be infected with strains resistant to antitubercular drugs.

HIV infection has been found to be an independent risk factor for drug resistance in tuberculosis.[17] In United States and other developed countries an alarming multiple drug resistance has recently been reported in HIV infected individuals. In some of the outbreaks the organism has been reported to be resistant to the two important drugs, rifampicin and pyrazinamide; in other cases to as many as seven drugs (INH, rifampicin, streptomycin, ethambutol, ethionamide, kanamycin and rifampicin). Poor compliance in this category of patients for various reasons such as drug abuse has been blamed for this. Transmission of these resistant bacilli to other HIV infected people, their family members and health workers has been reported, thus increasing the load of primary drug resistant cases in the community. The treatment in these cases has to be extended for longer duration increasing, the cost by 30-35 times and the risk of toxicity, treatment failure relapse and mortality.

Treatment of MDR Cases.

When there is no response to chemotherapy in a patient it is possible that the initial strain was multiple drug resistant i.e., primary resistance; and if there is an initial response followed by bacteriological failure while chemotherapy is still on it is probably because of acquired drug resistance or additional resistance has emerged. In both the situations, patient's treatment history should be carefully reviewed and if facilities are available drug resistance tests should be done in a good quality control laboratory.

In all the MDR TB cases first line drugs, streptomycin, INH, rifampicin, pyrazinamide and ethambutol are used only if they have not been used earlier and second line drugs, capreomycin, kanamycin, amikacin, ethionamide, prothionamide, para aminosalicylic acid and cycloserine should be used. The retreatment should consist of a minimum of five drugs of which atleast two , preferably three should be new drugs that do not have cross resistance with the drugs which have been used before. Single drug should not be added to the regimen that has failed. Chemotherapy should then be continued for atleast 12 months from the time the sputum smear becomes negative. Some of the newer experimental drugs are 1) Quinolones- ciprofloxacin, ofloxacin, sparfloxacin, 2) Rifamycin derivatives- rifabutin, rifapentine, 3) Macrolides- roxithromycin, clarithromycin, azithromycin, 4) Betalactam antibiotics with clavulinic acid, 5) cephalosporins- cefornide, 6) Oxazolidiones and 7) Azaquinone (Gangamycin).

WHO recommends that under programme conditions in countries where sources are limited treatment of patients with chronic tuberculosis should be given least priority. One option availalbe is to prescribe life long INH for such patients in the hope that this will diminish their infectivity and reduce the transmission of resistant organisms [12].

Difficulties in Treating Tuberculosis in Immunocompromised Patient

i) Side effects and toxicity

Although HIV infected patients with tuberculosis tolerate antituberculosis chemotherapy well, the incidence of drug toxicity

reported in them is more [8]. There are reports to indicate that rifampicin is not well tolerated. In 90% of the cases reactions occur during the first two months and in one study 18% patients required a change in therapy [8].

(ii) Drug Resistance (already discussed)

(iii) Drug Interaction

Superadded fungal infections are also common in these patients, and when they co-exist with tuberculosis, treatment is all the more difficult, as antifungal agents and antimicrobials are reported to have complex interaction [18]. Isoniazid and rifampicin reduce the serum concentration of ketoconazole and fluconazole. On the other hand ketoconazole inhibits the absorption of rifampicin from the gut and thus the two drugs taken together may lead to the failure of tuberculosis treatment.

iv) Treatment of non-tuberculous mycobacteria

The non tuberculous mycobacteria have heterogenous invitro antibiotic susceptibility patterns and generally do not respond to routine short course chemotherapy. Treatment regimens must be designed on the basis of specific species identification and knowledge about the nature and extent of infection as well as the integrity of hosts immune defence mechanism. While M.kansasii, M.marinum and M.xenopi are sensitive to many conventional antimycobacterial drugs like INH, rifampicin and ethambutol. M.fortuitum and M.chelonae are resistant to first line agents. They are often sensitive to second line drugs like doxycycline, macrolide antibiotics such as erythromycin, clarithromycin, imipenem and antibiotic such as amikacin, cefoxitin, trimethoprim-sulphamethoxazole and quinolones.

M.scrofulaceum is one of the most resistant organism and in this surgical excision is the treatment of choice. MAC infected individuals are recommended treatment with 3-6 drugs including conventional antimycobacterial agents with the addition of amikacin, ciprofloxacin, rifabutin, clofazimine, cycloserine, ethionamide, capreomycin, imipenem and other agents [19,20]. The outcome of treatment in these organisms however, rests on a pessimistic note.

v). Drug administration

It is reported that drug compliance in these patients is very poor and almost one third of the patients do not complete treatment [18] which also results in outbreaks with multi drug resistant strains.

The CDC has recommended Directly Observed Therapy Short Course (DOTS) which would be a closely supervised treatment that would ensure proper intake of drugs resulting in fall in the pool and finally control. However, the problem in developing countries in contrast to the developed countries is not so much of compliance as that of availability of drugs. Unless this problem is solved the success of DOTS is obviously questionable.

Drug Supports

Immunotherapy

There are now reports that immunological adjuvent therapies like interferon, interleukin 2, TNF may be of value in HIV infected individuals with tuberculosis. One such therapy which is rapidly gaining ground is immunotherapy with killed suspension of M.vaccae - a rapidly growing non pathogenic mycobacterium. J.L. Stanford [22], the pioneer of this therapy is of the opinion that if given along with chemotherapy it not only holds the possibility of better treatment and shorter regimens for newly diagnosed patients but also better prospects for chronic and drug resistant cases. Shorter regimens mean better compliance and less chances of bacilli developing drug resistance. The patient feels better faster and is also less infectious - a point which is very important in multidrug resistant tuberculosis. (Refer to original article by Stanford "Immunotherapy for Tuberculosis - Myth of Reality" published in this proceedings)

Immunotherapy is based on the principles of stimulating host defence mechanism, particularly the TH_1 cells to recognize mycobacterial antigens and eliminate the dormant population of persisting mycobacteria. The actively metabolizing bacilli are killed by the very first dose of rifampicin and INH. It is the small percentage of bacilli surviving the initial attack, either because of their lack of susceptibility to treatment used or because of their very low metabolic activity inside or outside the macrophages, that need to be eliminated. Immunotherapy does just that and therefore as an adjunct to chemotherapy is effective not only in drug sensitive situa-

tions but also in drug resistant cases.

Studies with M.vaccae carried out in Kuwait [23], Argentina [24], Nigeria [25], Romania and South Africa [22] have all shown important benefits to the patients.

A study from Iran [26] on cases with multi drug resistant tuberculosis and two years follow up has reported that addition of as many as four injections of M.vaccae eleven of the 42 patients were successfully cured ($p<.0001$). According to Stanford and Stanford [22] in chronic patients who had had treatment for as long as 81 to 118 months, cure rate of 22 to 30% could be achieved by giving as many as 6 injections.

Immunotherapy in HIV seropositive patients with tuberculosis

In a study carried out in Kano, Nigeria, Stanford et al [26] demonstrated in two years follow up a significant difference in mortality ($p < 0.0005$) among HIV seropositive patients who received immunotherapy with M.vaccae against those who did not. Out of the 8 subjects with immunotherapy followed up for two years all were alive as against 1 out of the nine in the placebo group. Moreover all the subjects with immunotherapy showed distinct clinical improvement with regression of lymphnodes.Sputum was also negative in all except one. Further studies in seropositive subjects are in progress in Durban, South Africa and North Hampshire USA(personal communication J.L. Stanford) which are likely to provide more data on this.

Prophylaxis

Chemoprophylaxis

It appears that INH prophylaxis is beneficial in HIV infected individuals. The WHO guidelines [28] for isoniazid preventive therapy (IPT) in HIV positive individuals emphasises that IPT should only be considered for HIV infected people with a positive tuberculin skin test (>5mm) who do not have active TB. Only when skin test is positive and active TB has been excluded by x-ray and culture should isoniazid be given at the daily dose of 5mg. per kg. up to a maximum of 300 mg for 6-12 months.

Persons receiving preventive therapy should be monitored monthly for adherence, toxicity and the development of active tuberculosis. Hepatotoxicity is one of the most common side effects specially in adults over 35 years and can be fatal. However,

in HIV positive people the benefits of IPT generally outweigh this risk.

At present there is no accepted standard for prophylaxis of non tuberculous mycobacterial infection in individuals believed to be at risk of developing disease. Efforts are on to find a safe and cost effective drug that may prevent infection due to MAC and other mycobacteria.

Immunoprophylaxis :

BCG is a live attenuated strain of M.bovis. Though disseminated BCG disease is rare it has been reported in subjects with impaired CMI, like infants born with genetic immunodeficincy syndromes, children born with HIV infection or immunized HIV infected adults. Because of this risk in immunocompromised individuals WHO [29] guidelines are to withhold BCG vaccination in cases of HIV infection especially where the risk of TB is low. Similarly, it should not be given to subjects with congenital immunodeficiency syndromes, haematologic neoplasms, generalised malignancies, patients receiving corticosteroids or immunosuppressive therapy.

Table - 1

Conditions Associated with Increased Rates of Mycobacterial Infection

Mycobacterium tuberculosis	Non tuberculous mycobacteria
Crowded living conditions	HIV infection
HIV infection	Neoplastic diseases
Neoplastic diseases	Immunosuppressive therapy
End stage renal disease	Chronic obsttuctive lung disease
Immunosuppressive therapy	Invasive procedures/
Silicosis	Percutaneous inoculation with
Upper gastrointestinal surgery	contaminated material
Malnutrition	End stage renal disease
Alcohlism	
Diabetes mellitus	Other congenital/acquired
Ageing	defects of CMI
Other congenital/acquired	
defects of CMI	

Table - 2

Category 1: New cases of smear positive pulmonary tuberculosis and new diagnosed seriously ill patients with severe forms of tuberculosis .
TB SCC treatment for new adult cases

Pretreatment body weight (kg)	INITIAL PHASE					
Adults	**2 months DOT**					
> 33kg	isoniazid and rifampicin H: 100mg; R:150mg 3 tablets daily	AND	pyrazinamide Z:500mg 3 tablets daily	AND	ethambutol E:400mg 2 tablets daily	OR streptomyc Powder 750mg injection d
>50kg	isoniazid and rifampicin H:150mg; R:300mg 2 tablets daily	AND	pyrazinamide Z:500mg 4 tablets daily	AND	ethambutol E:400mg 3 tablets daily	OR streptomyc powder 1g base in injection d
	CONTINUATION PHASE					
	4 months DOT			OR	**not DOT** 6 months	OR **6 months D** Immunocomprom but HIV negativ
>33kg	isoniazid and rifampicin H:100mg, R:150mg 3 tablets 3 times weekly **	AND	isoniazid H:300mg 1 tablet, 3 times weekly		isoniazid and ethambutol H:150mg E: 400mg 2 tablets Daily	isoniazid a thiacetazo H:300mg T: 150mg 1 tablet D
> 50 kg	isoniazid and rifampicin H:100mg, R:150mg 4 tablets 3 times weekly	AND	isoniazid H: 300mg 1 tablet, 3 times weekly		isoniazid and ethambutol H:150mg E:400mg 2 tablets daily	isoniazid thiacetazo H:300mg T:150mg 1 tablet da

*This category includes patients with tuberculous meningitis, disseminated tuberculo tuberculous pericarditis, peritonitis , bilateral or extensive pleurisy, spinal disease with n rological complications, smear negative pulmonary tuberculosis with extensive parenc mal involvement, and intestinal or genitourinary tuberculosis.

** When isoniazid+ rifampicin is given daily in the continuation phase, the drug doses the same as in the initial phase.
Source: WHO TREATMENT GUIDLINES 1993 [12]

Table -3

ategory 2: Relapse and treatment failure.

re-treatment for adults

etreatment dy weight g)	INITIAL PHASE							
dults	3 months DOT						First 2 months only	
33kg	isoniazid and rifampicin H:100mg;R:150mg 3 tablets daily	AND	pyrazinamide Z:500mg 3 tablets daily	AND	ethambutol E:400mg 2 tablets daily	AND	streptomycin powder 750 mg injection daily	
50kg	isoniazid and rifampicin H:150mg;R:300mg 2 tablets daily	AND	pyrazinamide Z:500mg 4 tablets daily	AND	ethambutol E:400mg 3 tablets daily	AND	streptomycin powder 1g base in vial injection daily 750 mg	
	CONTINUATION PHASE							
	5 months DOT							
33kg	isoniazid and rifampicin H:100mg.R:150mg 4 tablets 3 times weekly	AND	isoniazid H:300mg 1 tablet, 3 times weekly	AND	ethambutol E:400mg 4 tablets 3 times weekly			
50kg	isoniazid and rifampicin H:100mg.R:150mg 4 tablets 3 times weekly	AND	isoniazid H:300mg 1 tablet 3 times weekly	AND	ethambutol E:400mg 4 tablets 3 times weekly			

ource: WHO TREATMENT GUIDELINES [12]

Table - 4

Category 3: Pulmonary smear negative tuberculosis with limited parenchym involvement and extra pulmonary tuberculosis

Pretreatment body weight (kg)	INITIAL PHASE			
Adults	**2 months DOT**			
>33kg	isoniazid and rifampicin H:100mg;R:150mg 3 tablets daily	AND	pyrazinamide Z:500mg 3 tablets daily	
>50kg	isoniazid and rifampicin H:150mg; R:300mg 2 tablets daily	AND	pyrazinamide Z:500mg 4 tablets daily	
	CONTINUATION PHASE			
>33kg	isoniazid and rifampicin H:100mg, R:150mg 3 tablets 3 times weekly	AND	isoniazid H: 300mg 1 tablet, 3 times weekly	isoniazid and thiacetazo H:300mg, T:150mg 1 tablet daily
>50kg	isoniazid and rifampicin H:100mg, R:150mg 4 tablets 3 times weekly	AND	isoniazid H: 300mg 1 tablet, 3 times weekly	isoniazid and thiacetazo H:300mg, T:150mg 1 tablet daily

Source: WHO TREATMENT GUIDELINES 1993 [12]

Table - 5

Recommended Treatment Regimens for Patients who have Tuberculosis with or without HIV Infection*

CLINICAL SITUATION	WITH HIV INFECTION	WITHOUT HIV INFECTION
Initial Therapy		
No suspicion of drug resistance	Isoniazid,rifampicin,pyrazinamide	Isoniazid,rifampicin, pyrazinamide
Possible drug resistance	Isoniazid, rifampicin,pyrazinamide, ethambutol	Isoniazid, rifampicin, pyrazinamide ethambutol
Long-term therapy		
Drug susceptible organisms	Isoniazid,rifampicin,pyrazinamide for 2 mo and isoniazid, rifampicin for 7 mo, or for 6 mo after cultures are negative,whichever is longer	Isoniazid, rifampicin, pyrazinamid for 2 mo and isoniazid, rifampicin for 4 mo
Isoniazid resistance or intolerence	Rifampicin, ethambutol for 18 mo, or for 12 mo after cultures are negative, which ever is longer	Rifampicin, ethambutol for 12 mo
Rifampicin intolerence ethambutol	Isoniazid, pyrazinamide, ethambutol for 18 to 24 mo, or 12 mo after cultures are negative, whichever is longer	Isoniazid, pyrazinamide for 18 to 24 mo

* The recommendations are based on those of the CDC [7], Davidson [15], and the American Thora Society [4]

References :

1. Clinical Approach to Infection in the Compromised Host. Robert H. Rubin and Lowell S. Young. 3rd Edt. 1994 Plenum Medical Book Co. N. York and London.
2. Selwyn P A, Hartel D, Lewis V A et al. A Prospective Study of the risk of Tuberculosis among Intravenous drug users with HIV infection. N Eng J Med 1989; 320: 545-50.
3. Daley C L, Small P M, Schecter G F et al. An outbreak of Tuberculosis with accelerated progression among persons infected with human immunodeficiency virus. N Engl J Med 1992; 326: 231-5.
4. Di Perri G, Cruciani M, Danzi M C et al. Nosocomial epidemic of active tuberculosis among HIV infected patients. Lancet 1989; 2: 1502-4.
5. Centers for Disease Control and Prevention. Estimates of future global tuberculosis morbidity and mortality. MMWR 1993; 42: 961-4.
6. Horsburgh C R, Selik R M. The epidemiology of disseminated non tuberculous mycobacterial infection in the acquired immunodeficiency synderome (AIDS). Am Rev Respir Dis 1989; 139: 4-7.
7. Trener C, Hopewell P, Elias D et al. Human immunodeficiency virus infection in tuberculosis patients. J Infect Dis 1990: 162: 8-12.
8. Small P M, Schecter G F, Goodman P C et al. Treatment of tuberculosis in patients with advanced human immunodeficiency virus infection. N Eng J Med 1991; 324: 289-94.
9. D Cock K, Soro B, Coulibaly I et al. Tuberculosis and HIV infection in Sub Saharan Africa. JAMA 1992; 268: 1581-7.
10. Shafer R, Kim D, Weiss J et al. Extrapulmonary tuberculosis in patients with human immunodeficiency virus infection. Medicine 1991; 70: 384-96.
11. Perriens J, Colebunders R, Carahnnga C et al. Increased mortality and tuberculosis treatment failure rate among human immunodeficiency virus (HIV) seropositive compared with HIV seronegative patients with pulmonary tuberculosis treated with "standard" chemotherapy in Kinshasa, Zaire. Am Rev Respir Dis 1991; 144: 750-5.
12. Treatment of Tuberculosis. Guidelines for National Programmes. WHO 1993: 22-23.
13. Tuberculosis and human immunodeficiency virus infection. Recommendations of the Advisory committee for the estimation of tuberculosis (ACET). MMWR 1989; 38: 236-8, 243-50.
14. American Thoracic Society. Treatment of tuberculosis and tuberculosis infection in adult and children. Am Rev Respir Dis 1986; 134: 355-63.
15. Davidson P T. Drug resistance and the selection of therapy for tuberculosis. Am Rev Respir Dis 1987; 136: 255-7.
16. Charisson R, Schecter G, Theur C et al. Tuberculosis in patients with acquired immunodeficiency syndrome: clinical features, response to therapy and survival. Am Rev Respir Dis 1987; 136: 570-4.
17. Fieden T R, Sterling T, Publo-Mendez A et al. The emergence of drug resistant tuberculosis in New York City. N Eng J Med 1993; 328: 521-6.
18. Engelhard D, Stutman M R, Marles M I. Interaction of ketaconazole with rifampicin and isoniazid. N Eng J Med 1984; 311: 1681-3.
19. Etzkorm E T, Aldroundo S, McAllister C K, et al. Medical therapy of Mycobacterium avium-intracellulare pulmonary disease. Am Rev Respir Dis 1986; 134: 442-5.
20. Tsukamura M. Evidence that antituberculosis drugs are rarely effective in the treatment of pulmonary infection caused by Mycobacterium avium complex. Am Rev Respir Dis 1988; 137: 144-8.
21. Iseman M, Cohn D, Sbarbaro J. Directly observed treatment of tuberculosis. N Eng J Med 1993; 328: 576-8.

22. Stanford J L, Stanford C A. Immunotherapy for tuberculosis with M. vaccae. J Med Microbiol 1996; 44, 1: 24-8.
23. Bahr G M, Shaaban M A, Gabriel M et al. Improved immunotherapy for pulmonary tuberculosis with Mycobacterium vaccae. Tubercle 1990; 71: 259-99.
24. Vieira A, Domingo J, Valentini E, Hartopp R, Bottasso O A. A pilot study of immunotherapy with M. vaccae against Tuberculosis. Tubercle Lung Dis 1994; 75 Suppl 3: 47-8.
25. Onyebujoh P C, Abdulmumini, T, Robinson S, Rook GAW, Stanford J L. Immunotherapy for Tuberculosis in African conditions. Respir Med 1995; 89: 199-207.
26. Stanford J L, Onyebujoh P C, Rook GAW et al. Old plague, New plague, and a treatment for both? AIDS 1993; 7: 1275-77.
27. Farid R, Etemadi A, Mehvar M et al. Mycobacterium vaccae immunotherapy in the treatment of multi- drug- resistant tuberculosis: a preliminary report. Iranian J Med Sci 1994; 19: 37-9.
28. Cock K M De, Grant A, Porter J H D. Preventive therapy for tuberculosis in HIV infected persons: international recommendations, research, and practice. Lancet 1995; 345: 833-36.
29. WHO. Special Programme on AIDs and expanded programme on immunisation. Consultation on human immunodeficiency virus and routine childhood immunisation. Wkly Epidem Rec 1987; 62: 297-9.

Short Course Chemotherapy of Tuberculosis In Children

Pushpa Chaturvedi

Remarkable progress has been achieved in the chemotherapy of tuberculosis during the last few decades. One of the major break through has been the discovery of potent bactericidal drugs like Rifampicin (RIF) and Pyrazinamide (PZA) which when used along with the earlier available bactericidal drugs Isoniazid (INH) and Streptomycin (SM) have not only reduced the duration of therapy but drug regimens have been formed to prevent drug resistance.

Until 1945 there was no effective treatment. SM was introduced in 1945. The addition of Paraminosalicylic Acids (PAS) and INH followed closely. Though the prognosis improved but the morbidity and mortality was still unacceptable. This was due to the prolonged therapy required leading to irregular treatment and premature stoppage of drugs. Such default led to the emergence of drug resistant strains. [1, 2] It was thought that with the availability of powerful antimycobacterial drugs needing short regimens we will be able to overcome this problem. But unfortunately there is evidence to show that both initial and acquired resistance to antitubercular drugs is on the rise. [3, 4] There is a global concern about rising emergence of drug resistance and HIV infection. In fact drug resistant tuberculosis is on the rise even in HIV negative patients.

Current Drugs and their Efficacy:

Understanding of the biological characteristics of M. tuberculosis has helped in formulating drug regimens for optimum effect [5, 6]. Different subpopulations of M. tuberculosis have been identified in human disease (Table 1). There is a highly active large extracellular bacillary load present in the walls of cavitatary lesions, where condi

tions of high oxygen content and neutral or alkaline pH are favourable for their growth. INH, RIF and SM are the most effective drugs for this bacterial population. In closed caseous lesions oxygenation is poor hence the organisms grow slowly and intermittently, RIF and INH are useful here. PZA is bactericidal in relatively acidic intracellular environment of macrophages where the organisms are likely to become "Persistors," and in areas of acute inflammation.

The extent of bactericidal and sterilizing activity and prevention of resistance varies for different antitubercular drugs (Table-II). The intensity of bactericidal activity determines the rate at which viable tubercle bacilli disappear from the sputum during the first few days of chemotherapy. It is the sterilizing activity of a treatment regimen that determines the duration for which it must be taken. INH has the best early bactericidal activity. RIF and PZA have a high sterilizing action. INH, RIF, PZA Ethambutol (EMB) and SM are hightly suitable for intermittent use [7] . INH and RIF are effective in preventing the emergence of resistance. Thiacetazone (TCZ) is bacteriostatic and is used along with INH to inhibit emergence of resistance to INH in regimens that do not contain RIF. Thus five drugs INH, RIF, PZA, SM and EMB are currently regarded as essential drugs in the management of tuberculosis. TCZ is also widely used in many developing countries where its low price is considered to offset its relative lack of potency. [8]

Short Course Chemotherapy (SCC):

The modern SCC aims at rapid bactericidal and sterilizing action and reducting the duration of therapy. The basis of combination chemotherapy is to reduce acquired drug resistance. Irregular and inadequate drug administration or less potent regimens are responsible for therapeutic failures and emergence of drug resistance.

The SCC regimens was first tested in adults. The efficacy of the regimens could be evaluated more objectively in adults by sputum conversion rates, as 95% of tubercular disease in children aged 0-9 years and 90% of disease in those aged 10-14 years is non-infectious [5]. The first report of successful SCC in adults came from East Africa [9]. SCC is now an accepted treatment for most forms of tuberculosis.

Pecularities of childhood tuberculosis make it more amenable to SCC with better outcome. This is because many children with pulmonary tuberculosis are either asymptomatic or have less extensive disease and rarely cavitatory lesions as compared to adults, but they have a higher rate of occurrence of extra-pulmonary tuberculosis. Both are associated with a low bacillary population. They also have less secondary bacterial resistance, lack of primary drug resistance, tolerate higher drug doses and have low incidence of adverse reactions.,[5,10]

On review of efficacy of various regimens the following are important principles to be followed while giving SCC:

1 Two bactericidal drugs to which the bacteria are sensitive must be in the regimen.

2 RIF & PZA must be included in the first 2 months. PZA should not be replaced by SM or EMB in this phase except under special situations.

3 INH must be included in the full duration of the regimen, unless resistance is documented. Currently recommended dose is 5 mgm per kg per day[5].

4 In an apparently failing regimen, always add two drugs, never a single drug.

5 The commonest cause of therapeutic failure, relapse and drug resistnace is non-compliance of the drug regimen.

SCC Regimens in Childhood Tuberculosis:

The earlier conventional treatment using INH & TCZ/PAS along with SM for first 2 months was recommended for 18-24 months. This prolonged therapy lead to very poor compliance, specially when children have to depend upon parents for regularity of medication. After discovery of RIF the duration was curtailed to 9 months using INH and RIF daily for 2 months and either daily or twice weekly for the remaining 7 months with good results.[11]

With the success of 6 months SCC regimens in adults it seemed logical to apply this development to childhood tuberculosis. The first trial of 6 month regimen in children was carried out by Dingley, [12] following which a number of paediatric studies of 6 month therapy have been reported using slightly different regimens, all containing

atleast three durgs in the initial phase of treatment usually INH, RIF and PZA[13 to 19]. Directly observed intermittent therapy in the continuation phase was as effective as self administered daily therapy. Intermittent therapy for the entire 6 months has success rate equivalent to daily therapy for initial 2 months.[14,19] Following are the regimens recommended in different categories of childhood tuberculosis.

1. Asymptomatic tuberculin positive child with history of close contact with a sputum positive case, or with recent documented tuberculin conversion are at a high risk of developing tuberculosis.[5] In a country with a high prevalence rate of tuberculosis a large tuberculin reaction cannot be ascribed to BCG vaccination, further tuberculin reactivity tends to wane significantly after vaccination.[20] Recommendations for these children are varied from INH alone for 6 months[5] to INH and TCZ for 3 months.[20] A recent report has shown good results with the use of INH and RIF for 3 months, but results, were best when INH, RIF and PZA were given for 1 month.[21]

2. Pulmonary Primary Complex - A combination of INH and RIF daily or intermittently for 6 months is recommended.[20, 22] International Union against Tuberculosis and Lung disease (IUATLD) recommends INH, RIF and PZA for 2 months followed by INH and RIF for 4 months.[5]

3. Progressive Pulmonarydisease,endobronchial tuberculosis and pleural tuberculosis are treated with three drugs INH, RIF and PZA for first 2 months followed by INH, RIF for 4 months. If INH resistance is suspected or proven EMB or SM can be added for the initial two months followed by 4 months of INH, PZA and EMB (or SM), followed by 4 months of INH and RIF thus giving a 10 months course.[5]

4. Tuberculosis of central nervous system, miliary tuberculosis and disseminated tuberculosis :- INH, RIF and PZA all cross the blood brain barrier without difficulty. EMB and SM diffuse well during acute meningeal inflammation. Most studies recommend a total duration of therapy between 9 - 12 months.[5,6,20,23] Four drugs SM, INH, RIF and PZA are given for initial 2 months followed by two drugs INH and RIF for the remaining 10 months[5,6]

RIF can be substituted by EMB, also during the initial and continuation phase. INH or RIF can be given twice weekly instead of daily to reduce hepatotoxicity [20,23]. When curtailing the duration to 9 months after initial 2 months of four drug therapy, three drugs INH, RIF and EMB can be given for 4 months, followed by INH and EMB for remaining 3 months .[20,23]

5. Extrapulmonary tuberculosis at other sites including tubercular lymphadenitis are treated just like progressive pulmonary disease. [18,19] Some recommend that bone and joint tuberculosis should be treated in the same lines as for tubercular meningitis.[6]

Indications of Corticosteroids in tuberculosis:

Corticosteroids as adjunctive therapy are beneficial in tubercular meningitis, pericardial disease, large pleural effusion with significant symptoms and endobronchial tuberculosis.[6] The usual dose is prednisolone 2mg per day orally daily for 4-6 weeks and then tapered off.

Drug Resistance :

The incidence of drug resistant tuberculosis is on the rise.[3,4] Factors responsible for this are poor drug compliance,uncontrolled and irrational use of drugs over the counter and improper dose and drug regimens prescribed by doctors. Primary resistance to SM or INH is most common, whereas it is rare with RIF and PZA. EMB or PZA should be added in initial phase in situations where there is increased risk of primary INH resistance. The prevalence of primary INH resistance in India is reported to be 10.6%.[24] American thoracic society recommends an initial four drug regimen in areas where there is more than 4% initial resistance to INH. It is debatable if we need to follow this rcommendations in treating childhood tuberculosis in India.[26]

Compliance :

Non compliance is the major problem in tuberculosis control. Every effort must be made to emphasize the importance of drug compliance to the family members. If compliance is poor the child should be put on direct supervision of drug intake whether the regimen is daily or intermittent. This is easier said than done. It is nearly impossible to ensure that the patient will attend the drug distribution centre regularly for the medicines. Additionally this strategy cannot ensure intake of RIF on empty stomach for adequate bioavailability of drug.[27]

Thus to summarise multidrug and short chemotherapy in the treatment of tuberculosis using appropriately recommended regimens and proper drug doses has its rewards of early sterilisation of lesions and shortening the duration of therapy in the controlled clinical trials reported. But at the same time without proper drug compliance the purpose is defeated, with increase in incidence of drug resistant tuberculosis. In developing countries lack of health conciousness, poor knowledge about the disease process, lack of supervised therapy in field conditions, non availability of drugs in tubercular centres, poverty leading to inability to purchase drugs, need to be tackled at a war footing, with emphasis on case finding and appropriate chemotherapy and drug compliance for effective control of tuberculosis.

TABLE - I

Drug Efficacy in different Bacterial Populations

Drug	BACTERIAL POPULATION			
	Active Extra cellular	Semidormant caseous	Dormant intra cellular	Resistant Mutants
INH	+++	++	+	+++
RIF	+++	+++	++	+++
PZA	-	-	+++	-
SM	+++	-	-	++
EMB	++	-	++	++

TABLE - II

DRUG ACTIVITY

Activity	Good	Moderate	Little or ml
Bactericidal activity	INH	RIF, EMB	SM, PZA, PCZ
Sterilizing activity	RIF, PZA	EMB	INH,SM
Inhibitors of acquired resistance	INH, RIF	SM, EMB	PZA, TCZ

ml = minimal

TABLE - III

Drug Doses in Tuberculosis (mgm per g)

Drug	Daily	Maximum	Intermittent*	Maximum
INH	5-10	300	20-40	900
RIF	10	600	15	600
SM	15-20	1000	25-30	1000
PZA	25(2 months) 15	2000	50-70	2000
EMB	25 (2 months) 15	2500	50	2500
TCZ	2-4	150	-	-

* Twice weekly

References

1. Indian Council of Medical Research. First drug resistance investigations. Ind J.Med Res 1968; 3: 1617- 19
2. Indian Council of Medical Research. Second drug resistance investigation Ind. J Med Res 1969, 57 : 823-25
3. Chandrasekaran S, Chauhan MM,Rajalakshmi R, Chaudhary K., Mahadev B. Initial drug resistance to antituberculosis drugs in patients attending urban district tuberculosis centre. Ind J Tub 1990, 37 : 215-216
4. Gupta P R, Singhal B, Sharma TN, Gupta RB. Prevalence of initial drug resistance in tuberculosis patients attending a chest hospital. Ind J Med Res(A) 1993, 97: 102-103.
5. A statement of the scientfic committees of the IUATLD tuberculosis in children. Guidelines for diagnosis, prevention and treatment. Bull Int Union Tuberc Lung Dis 1991, 66: 61-67.
6. Committee of Infectious Diseases. Chemotherapy of tuberculosis in infants and children Padiatrics 1992,89:161-165.
7. Mitchison DA, Ellard G A, Grosset J H. New antitubercular drugs for the treatment of mycobacterial diseases in man. Brit Med Bull 1988, 44: 757-774.
8 Essential Drugs-Tuberculosis a persistant but treatable disease. WHO Drug Information 1990, 4: 30-41.
9. East African/British Medical Research Councils. Results at 5 years of controlled comparision of a 6 month and a standard 18 month regimen of chemotherapy for pulmonary tuberculosis. Am Rev Respir Dis 1977, 116: 3-8.
10. Strake JR. Multidrug therapy for tuberculosis in children. Pediatr Infec Dis J. 1990, 9:785-793.

11 Abernathy RS Dutt AK. Stead WW. Moers DJ. Short course chemotherapy for tuberculosis in childhood-paediatrics 1983, 72: 801-806.
12 Dingley HB Short term chemotherapy for tuberculosis in children. Indian J Tuberc 1982, 29: 48-54.
13 Pelosi F. Budani H. Ruberstien C et al.Isoniazid, rifampicin and pyrizinamide in the treatment of childhood tuberculosis with duration adjusted to the clinical status. Am Rev Respir Dis 1985, 131 (suppl) A229.
14 Varudkar BL. Short course chemotherapy for tuberculosis in children. Indian J Pediatr 1985;52:593-597.
15. Starke J R, Taylor -Watts KT. Six month chemotherapy of intrathoraccic tuberculosis in children. Am Rev Respir Dis 1989, 139(suppl) : A 314-318.
16. Medical Research Council Tuberculosis and chest disease Unit. Managemant and outcome for chemotherapy in childhood tuberculosis. Arch Dis child 1989, 64:1004-1012.
17. Khubchandani RP. Kumta NB, Bharucha NB et al. Short course chemotherapy in childhood pulmonary tuberculosis. Am Rev Respir Dis 1990, 141(suppl), A 338-340.
18. Bicolulph J. Short course chemotherapy for childhood tuberculosis. Padiatr Infect Dis J 1990, 9-:794-801.
19.Kumar L, Dhand R,Singhi PD, Rao KLN, Kataria S. A randomized trial of fully intermittent and daily followed by intermittent short course chemotherapy for childhood tuberculosis. Padiatr Infect Dis J1990, 9:802-806.
20.Seth V. Diagnosis and treatment of tuberculosis. An overview in tuberculosis in children 1st Edn Seth V. Puri RK, Sachdev HPS . Indian Academy of Paediatrics 1991; 8-52.
21. Gupta D K, Kumar R, Nath N, Kothari A K-Chemoprophylaxis in high risk children -Analysis of 8 years follow up : preliminary report. Ind J Tub 1993, 40: 125-127.
22.Jacobs R F, Abernathy R S . The treatment of tuberculosis in children. Padiatr Infec Dis J 1985,4:513-517.
23. Ramachandran P. Duraipandian M, Nagarajan M. Three chemotherapy studies of tubercular meningitis in children. Tubercule 1986, 67: 17-29.
24.Grange J M Drug resistance and tuberculosis elimination. Bull Int Union Tuberc Lung Dis 1990, 65: 57-59.
25.Official statement of American thoracic Sociaty,Endorsed by American Academy of Paediatric April 1993. Treatment of tuberculosis and tuberculosis infection in adults and children. Am J Resp Crit Care Med 1994, 149:1359-1374.
26. Seth V Chemotherapy of tuberculosis. Ind Padiatr 1996, 33: 146-147.
27. Kapoor SC. Chemotherapy-New Strategies ? Ind J Tub 1995, 42: 193-194.

IMMUNOTHERAPY FOR TUBERCULOSIS - *MYTH OR REALITY?*

J L Stanford and C A Stanford

Introduction

Let there be no doubt, immunotherapy for tuberculosis works and is here to stay for the benefit of patients. It is not an alternative to chemotherapy, but a valuable means of augmenting drug treatment. Not only does it holds the possibility of better treatment of patients with chronic and drug-resistant diseases, but it should lead to shorter regimens for newly diagnosed patients and reduces the chances of bacilli developing drug resistance.

Studies have been carried out using a suspension of killed mycobacterium vaccae as an immunotherapeutic in Africa, Asia, Europe and South America, all showing potential improvements over existing regimens. A Major phase III trial for regulatory purposes, which it is hoped will lead to granting a product licence, has just been completed in South Africa and the analysis of data will be carried out early in 1997.

The need for immunotherapy in tuberculosis

The current status of tuberculosis as a global emergency is chiefly due to the length and cost of treatment, leading to patients defaulting from treatment. The best available chemotherapy has to be taken for 6 months before success can be almost guaranteed. Where such treatment is too expensive, longer regimens of cheaper drugs have to be used. These cheaper drugs often have minor side effects which becomes unacceptable to many patients before treatment has been completed , making the situation worse. It is the combination of the

length of treatment and minor side effects that are directly responsible for patients defaulting from treatment, though this usually occurs after the 2 months intensive phase of chemotherapy has been completed. Such non-compliance leads to relapse, infection of further people and the development of drug resistance.

What is needed is not a new drug - this would be very unlikely to shorten the length of treatment and within a short time bacilli would be selected with resistance to it and we would be back where we are now. There are enough antituberculosis drugs already, what is needed is a drastic new look at the treatment of tuberculosis and the development of effective combination of chemotherapy with immunotherapy.

How chemotherapy works

Antituberculosis chemotherapy works directly on the bacilli, either slowing down their replication - bacteriostatic activity, or killing them - bactericidal activity. Bacteriostasis is effective by blocking certain metabolic pathways apparently making bacilli more susceptible to macrophage killing mechanisms. Bactericidal drugs works by blocking essential metabolism or by disrupting cell envelop structure and work best on rapidly metabolising organisms. Live, but resting bacilli are resistant to both types of drug action, though this resistance is lost once metabolism is speeded-up.

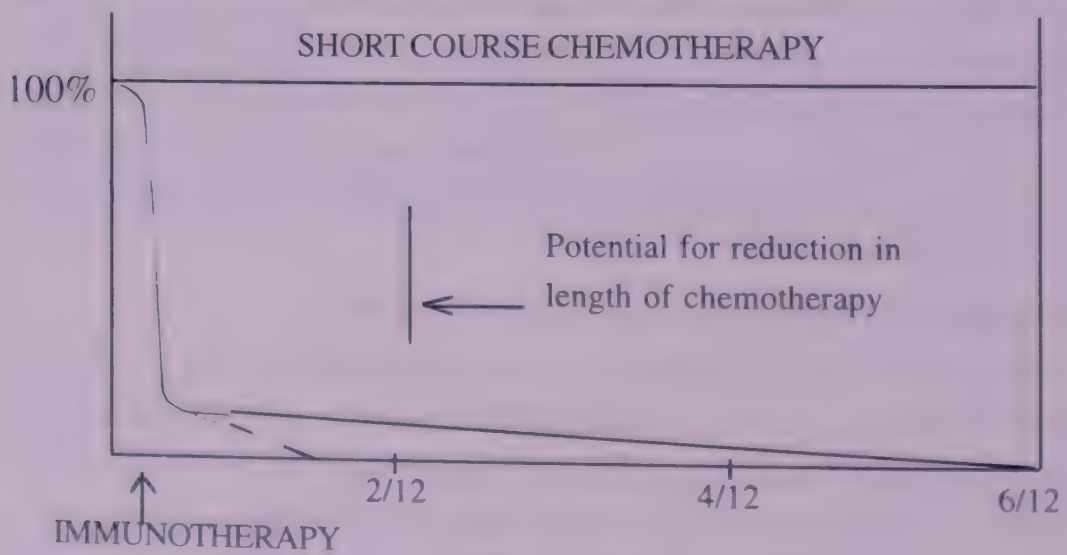


Figure 1

Diagram illustrating the eradication of live tubercle bacilli from the tissues of a patient with tuberculosis during modern standard short-course chemotherapy and the way this can be modified by the addition of immunotherapy.

The response of tubercle bacilli in tuberculosis patients to chemotherapy is a combination of 2 curves, one for the elimination of actively metabolising organisms, known as the **early bactericidal action (EBA)** of drugs, and the other for the elimination of the dormant, or semi-dormant bacilli, known as the **sterlising action** of the drugs. As shown in Figure 1, the early bactericidal action occurs in the first week or so of chemotherapy, during which the number of living bacilli in the tissue of the patient fall to 5% or so of their starting number . All the remaining months of treatment are needed to ensure adequate antibacterial levels when the 5% of persisting live bacilli begin to metabolise fast enough for the drugs to have their sterlising effect. Of course, superimposed on this is are variables such as the initial drug susceptibility of the strain and the pathological anatomy of the disease, such as the presence of thick walled cavities, extensive fibrosis, etc., which may restrict early bactericidal activity. Drugs also differ in the degree of the two effects that they may show.

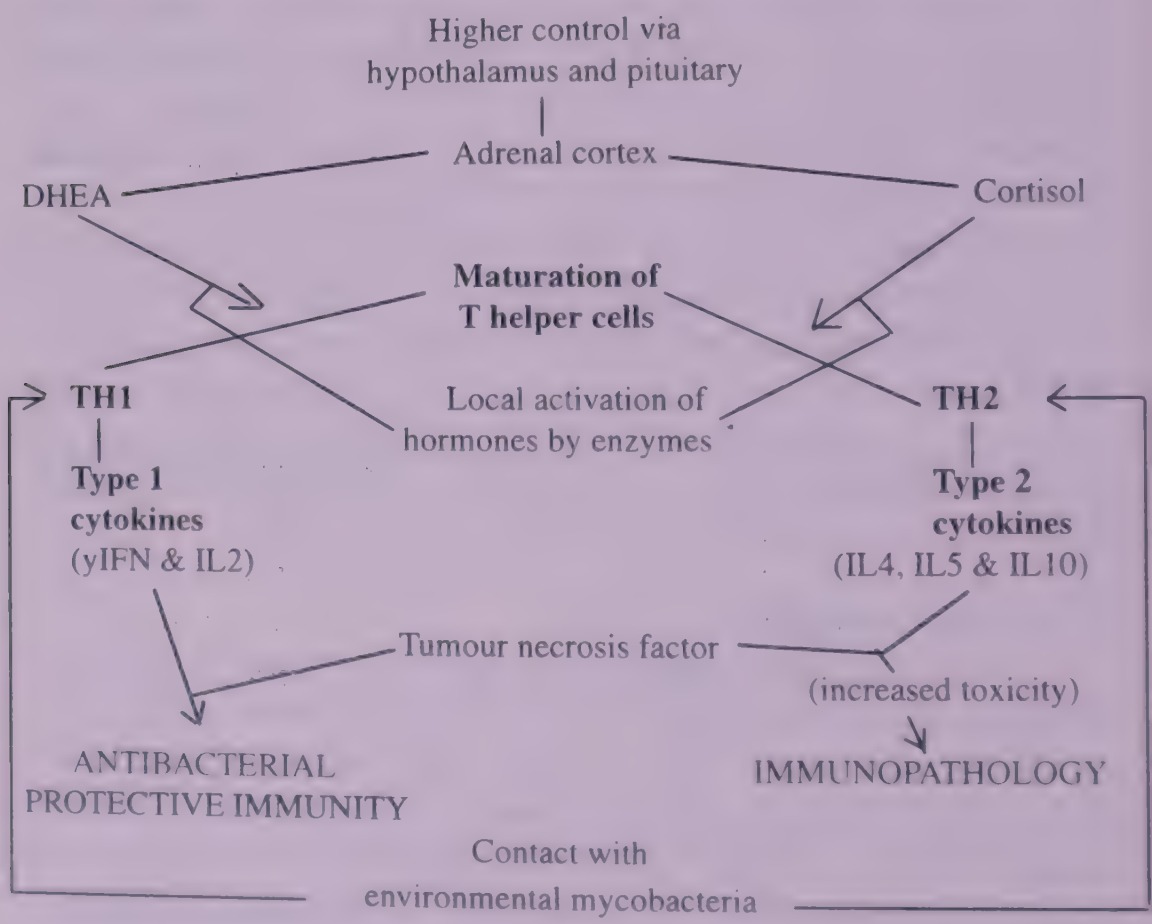
Nearly every patient complies with treatment long enough for the early bactericidal activity of the drugs and defaulting occurs during the phase of tissue sterilisation. Thus non-compliance is not usually followed by rapid relapse, but the passage of months, during which the semi-dormant persisting live bacilli reactivate and multiply up to the numbers needed to continue the disease process. Each time this occurs, there is a greater opportunity for naturally occuring drug-resistant bacilli to have been selected out to make up a larger share of the infecting population.

Multi-drug resistance

Modern evidence suggests that far from being the relatively dysgonic and disadvantaged bacilli with low catalase activity that used to be associated with isoniazid resistance, many drug-resistance strains replicate as fast and are as virulant as drug sensitive strains. Nowadays, most strains resistant to isoniazid and rifampicin produce normal amounts of catalase and seem to have the ability to very rapidly acquire resistance to additional drugs. Thus the ogres of 10-and 11 drug resistance of the literature easily becomes reality. With this situation already with us, it can be seen why just a new drug is not the answer to the problem.

How immunotherapy would have to work

Before or during the course of development of clinical tuberculosis, the optimum protective immune mechanisms becomes perturbed and relapsed with mechanisms best adapted to contain and limit existant disease, rather than to eliminate invading bacilli. This happens in response to overload with mycobacteria from the environment, especially when these belong to the slow-growing subgenus, or from the equivalent repeated exposure to inhaled tubercle bacilli. This may occur particularly at times when immunity is less than optimum, such as following infection with a virus or at times of emotional stress. This latter , although often recorded, has been difficult to explain until recent evidence became available showing that mental state, notably depressive psychosis, was accompanied by the immune status associated with susceptibility to tuberculosis.



(Figure 2)

Diagram illustrating the maturation of T helper (TH) cells into TH1 and TH2 and some of the mechanisms that may regulate the process.

For such a manifestly immunological disease, tuberculosis still defies the best efforts of those who would investigate it. Nonetheless, recent knowledge about the maturation pathways of helper T (TH) cells appears to have broken through and at least provided names to which mechanisms can be tied. Figure 2 shows the maturation of stem TH cells along the TH1and TH2 pathways, the principle cytokines produced and some mechanisms that may control them. One mechanism appears to be central and to work through the control of secretion of adrenal cortical hormones. The 2 important hormones, with their metabolites and derivatives that control TH maturation are cortisol and dehydroepiandrosterone (DHEA). When cortisol is in excess maturation towards TH2 is promoted and when DHEA predominates TH1 is promoted. The second layer of maturation control may be by the local production of enzymes capable of activating or deactivating the hormones. These control mechanisms themselves are influenced by cerebral functions and by environmental experiences. Thus susceptibility to disease following mental trauma may be explained and contact with environmental mycobacteria can either prime for protective efficacy of BCG vaccination or pre-empt it.

Table 1

The results of some trials of BCG vaccination against tuberculosis and leprosy.

Protection from tuberculosis		Protection from leprosy	
UK	80%	Uganda	80%
Alabama & Georgia	14%	Burma	18%
South India	0%		

The overall results in Table 1 probably represent a balance in each case between persons to whom immunity has been induced and those in whom increased susceptibility results. The effects of BCG, however may go beyond this since studies in The Gambia shows that mortality is lower in those developing clinical tuberculosis who have old scars of BCG, in comparision with those who do not have them. ($p<0.01$).

Table 1 also shows that BCG can be just as good a vaccine for leprosy as it is for tuberculosis, indicating the importance of the comman mycobacterial antigens, group i in Figure 3, in the induction of protective immunity. Further evidence for the likely

importance of these antigens is shown by the lack of skin-test responsiveness to them in tuberculosis patients.

Table 2

Results of quadruple skin testing in patients with pulmonary tuberculosis, and healthy, BCG vaccinated, Controls in Kuwait.

Reagents :	Tuberculin	Leprosin A	Scrofulin	Vaccin
PTb patients	86%	26%	24%	15%
	ns	p<0.00001		
Controls	94%	71%	.78%	76%

Groups of antigens

	i	ii	iii	iv
M. tuberculosis	//////	//////		//////////////
M. scrofulaceum	//////	//////		/////////
M. ulcerans	//////	//////		//////////
M. fortuitum	//////		//////	//////////////
M. chelonae	//////		//////	/////////
M. vaccae	//////			//////
M. leprae	//////			////////

(Figure 3)

Diagram showing the antigens of mycobacteria demonstrable by double diffusion in sonicate preparations of various species using high-titre rabbit antisera. Representatives of typical slow-growing species are shown at the top, representatives of typical fast-growers are below them and the somewhat unusual species, M.vaccae amd M.leprae are at the bottom. The common mycobacterial (group i) antigens shared by all species are shown on the left, the slow-grower associated (group ii) antigens are next to them, the fast-grower associated (group iii) antigens are shown next to them, and the species-specific (group iv) antigens limited to each individual species are shown on the far right.

The protective immune response appears to be through TH1 cells predominantly responding to group i, common mycobacterial antigens and producing, or stimulating the production of Type 1 cytokines. Important amongst these are Interleukin 2 (IL2), and Gamma interferon (IFNγ). These in turn are thought to derive mechanisms leading to the eradication of mycobacterial challenges. The mechanisms of susceptibility to mycobacterial disease and the

immunopathology caused appear to be through TH2 cells producing Type 2 cytokines including IL4, IL5, and IL10, in the presence of which, circulating and local tumor necrosis factor (TNF) becomes highly toxic. This toxicity leads to weight loss, fever, night sweats, and caseous necrosis of tissues at the sites of infection. Contact with different environmental mycobacterial species in variable quantities can promote either TH1 or TH2 and the superimposition of BCG leads to maximisation of whichever predominates at the time of vaccination. Thus BCG can lead to protection or susceptibility, explaining the different levels of protective immunity to tuberculosis and leprosy in different countries.

The same principles explain why BCG is ineffective as an immunotherapeutic for tuberculosis, although it might drive towards fibro-caseous forms of the disease. Just as a TH1 response to common mycobacterial antigens is essential for protective immunity, returning this response during the course of treatment for tuberculosis should be the basis of immunotherapy.

The selection of M.vaccae

It was postulated that the reason BCG was so effective against leprosy in Uganda might be because people living in the region were primed for this response by previous contact with some environmental influences - probably a mycobacterium. Thus an extensive series of bacteriological investigations of the Ugandan environment were carried out and a number of different mycobacterial species were identified. At that time it was thought that the organisms responsible would have some special relationship with leprosy bacilli, perhaps a sharing of additional antigens. To investigate this skin-test reagents were prepared from each of the isolated species, and these were used to assess the level of positivity in the Ugandan population, and to test in patients with various forms of leprosy. It was expected that skin reactions to the responsible organism would be negative in multibacillary disease, but positive in paucibacillary disease and in healthy close contacts of leprosy patients. These parameters were met best by M.vaccae and to a lesser extent by several other fast-growing species. Subsequently, when the soluble skin-test preparation leprosin A became available, about 95% concurrence of results

was achieved in Iran with the skin-test preparation of the selected strain of M vaccae from the Ugandan environment.

Animal experiments

When the selected M.vaccae was added to the drinking water of mice it was found to enhance the protective immune response following subsequent BCG vaccination. This same effect could be achieved by vaccinating mice with BCG to which 10^7 killed M.vaccae had been added. The dose of 10^7 was selected because this was the dose required by mice for optimal immune recognition of a subsequent injection of 10^4 M.leprae. Normally a minimum of 10^6 M.leprae have to be injected before mice can recognise their presence.

Initial investigations in man

Following safety tests in a few volunteers, studies were set up in 1978 in Leprosin A negative close contacts of leprosy patients. Some were randomised to receive BCG alone, others received BCG+10^7 M.vaccae, and those who had a scar of past BCG or who were tuberculin positive, were vaccinated with 10^8 M.vaccae alone. At follow-up skin-tests 8-10 years later, both the combination and M.vaccae alone were found to have induced Leprosin A positivity in 90% of recipients compared with 60% after BCG alone.

The breakthrough into immunotherapy for mycobacterial disease followed the demonstration by skin testing that adding skin-test reagent of M.vaccae to tuberculin (or an equivalent preparation) regulated out the necrotic aspect of the skin response in persons who would normally have shown it. This regulation occured both at the site of the injection of the mixed reagent and at the site of the injection on the other arm of the potentially necrotising reagent on its own. If this could happen between one arm and the other, why not from an arm to the lung?

Fractionation of the soluable M.vaccae reagent showed that different fractions were responsible for the local and distant regulatory effects.

Immunotherapy for tuberculosis

The immunotherapeutic is prepared from a special strain of M.vaccae grown on Sauton's agar, harvested and suspended in borate-buffered saline at pH8.0.At first the bacilli were killed by cobalt

irradiation, but subsequently autoclaving has been adopted. The reagent contains heat-stable fragments of antigens of group i and iv, adjuvant activity, and unidentified small molecules that may be of relevance to cell signaling.

Following dose-ranging studies, at first in leprosy and then in tuberculosis patients, the dose of 10^9 autoclaved M.vaccae was selected for investigation. Early investigations were carried out in London, Spain, Kuwait. Subsequently trials in tuberculosis have been carried out in The Gambia, Nigeria, Vietnam, Romania and India. A regulatory, phase III, trial has been carried out in South Africa for which the results are now being analysed and another is in progress in London.

In Northern Nigeria, where the quality and the availability of antituberculosis chemotherapy was appealing, a randomised and part-blinded study produced impressive results. As shown in Table 3, the chemotherapy available produced results hardly better than would have been obtained in the days before treatment, whereas the same chemotherapy plus an injection of M.vaccae given 7-14 days after starting chemotherapy produced results not much worse than would have been expected of a complete course of chemotherapy in that region.

Table 3

10-12 month follow-up of patients entering a randomised and partly blinded study of immunotherapy with M.vaccae in Kano, Nigeria, where the quality of chemotherapy was very poor.

	Immunotherapy group		Placebo group
Mortality	0/34	p<0.00001	19/47
Weight regain	7.9±1.0Kg	p<0.001	2.0±1.7Kg
Sputum AFB-ve	21/34(62%)	p<0.0001	4/26(14%)
Fall in ESR	42±8mm	p<0.001	15±9mm

When the results of 4 randomised studies of newly diagnosed pulmonary tuberculosis in which the patients received full course of chemotherapy in The Gambia, Vietnam and Romania are pooled, it can be seen that both cure-rate and survival are improved by the addition of an injection of M.vaccae though they are less impressive than the results obtained in Nigeria.

Table 4

Pooled data from 4 randomised and partly blinded studies in which patients took complete course of chemotherapy for newly diagnosed pulmonary tuberculosis.

	Immunotherapy group		Placebo group
Cure at end of chemotherapy	274/299(92%)	p<0.001	275/332(83%)
Deaths during chemotherapy	6/299(2%)	p<0.03	17/332(5%)

Switching immune predominance from type 2 to type 1 in the course of tuberculosis has effects beyond those on bacteria. The toxic effects of circulating TNF include loss of body weight, fever, night sweat, raised erythrocyte sedimentation rate (ESR), tissue death around lesions and cavitation of the lungs. These have been specially studied in Romania and the results are summerised in Table 5 for a group of patients with chronic pulmonary tuberculosis of more than 3 years duration.

A proportion of the chronic patients in Table 5 were infected with multi-drug-resistance tubercle bacilli (MDRTB), and their recovery was also improved by the immunotherapy. Since such bacilli differ little if at all in their antigenic characteristics, they should be just as susceptible to immunotherapy as are fully drug-sensitive bacilli. This proves to be so in the cases of primary MDRTB who all had history of less than 2 years of disease. Of 7 such patients 6 (86%) were cured and did not relapse over the next 2 years (the 7th was lost to follow-up, and may also have recovered). The results for patients with acquired MDRTB who all had histories of more than 2 years of disease are less impressive, with 11/50 (22%) being cured after from 1 to 4 injections of M.vaccae given at 2-6 month intervals. Most of these patients were receiving chemotherapy to which their bacilli had been shown to be resistant in vitro.

Table 5

Regain of body-weight, fall in ESR and closure of cavities in Romanian patients with chronic tuberculosis, receiving a full course of chemotherapy, with or without a single dose of M.vaccae given after 4 weeks of chemotherapy.

	Immunotherapy group		Placebo group
Mean body weight			
At start	56.3Kg		54.3Kg
After 6 months*	62.6Kg	p<0.005	56.7Kg
After 1 year	65.0Kg	p<0.002	57.5Kg
Weight increase	8.7Kg		3.2Kg
ESR			
At start	59mm		65mm
After 6 months*	29mm	p<0.01	39mm
After 1 year	19mm	p<0.0001	40mm
Fall in ESR	40mm		25mm
Cavitation[@]			
At start	55/62		42/48
Still with cavities			
After 6 months*	37/47		38/42
After 1 year	18/44	p<0.01	23/31
% reduction	59%		26%

*i.e. at the end of chemotherapy.
[@]numbers of patients with cavities.

Immunotherapy in tuberculosis of the HIV-infected

Among patients entering the study in Nigeria 19% were found to be seropositive for HIV1, by good fortune 8 were amongst those followed up after immunotherapy and 9 were followed up in the control group. The results achieved are shown in Table 6, and it can be seen that immunotherapy was just as effective as it was in the whole study (Table 3), but the HIV seropositive patients in the control group did somewhat less well than did HIV seronegatives.

Table 6

Results for HIV1 seropositive tuberculosis patients followed up in the Kano study.

	Immunotherapy group		Placebogroup
Numbers studied	8		9
With generalised lymphadenopathy at start of treatment	5/8		7/9
10-12 month follow-up			
Mortality	0/8	p<0.01	6/9
Sputum AFB +ve	1/8	p<0.03	3/3
Clinically improved	8/8	p< 0.001	0/3
Lymphadenopathy	0/8	p< 0.001	3/3
24 month follow-up			
Survival	8/8	p<0.001	?1/9

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Immunotherapy with M.vaccae for non-mycobacterial diseases

For two reasons M.vaccae may prove to be a valuable immunotherapeutic for many diseases not directly associated with mycobacterial infection. One reason is its nonspecific TH1 adjuvant action and the other is the sharing of some group i common mycobacterial antigens with human tissue antigens. In particular, the mycobacterial stress proteins share a variable amount of amino acid sequence homology with those of man. Notably the 65 kDa heat shock protein of BCG is atleast 50% homologous with the 60kDa heat shock protein of man. Stressed infecting mycobacteria produce their stress protein and these induce antibodies through TH2 mechanism that cross react with those of human stressed cells. Thus the stress of both the infecting organism and the host leads to antibodies that can bind to soluble bacteria stress protein, probably doing little harm to the bacilli, and also to stress proteins expressed on the surface of stressed host cells leading to their destruction. Once such an auto immune mechanism for tissue damage is in place it may constitute an important part of immunopathology.

Another important possibility in relation to auto-immune diseases is that contact or silent infection with mycobacteria could well be an aetiological factor. In support of this are the series of longterm sequelae of leprosy in which "bacteriologically cured" patients may develop arthritis similar to rheumatoid and reactional arthritis, anterior uveitis and develop cold fingers associated with a low-grade peripheral vasculitis. These sequelae respond to immunotherapy with M.vaccae.

Besides potential usefulness in identifiable autoimmune disease from rheumatoid arthritis to cardiovascular disease and schizophrenia, M.vaccae may be of use in the treatmant of cancer. The precise mechanism for such an action has not been fully elucidated, but it is likely that changed immune responses to stress proteins, TH1 predominance and perhaps regulation of IgA antibodies to bacteriomimetic carbohydrates on the surface of malignant cells, may hold the explanation. Table 7 is a list of non-mycobacterial diseases in which immunotherapy with M.vaccae may be of value. Those diseases for which we already have anecdotal evidence for a beneficial effect are marked by an asterik.

Table 7

Diseases in which immunotherapy with M.vaccae may prove to be of value

Anterior uveitis*	Osteoarthritis*
Atherosclerosis*	Pityriasis versicolor*
Cancer*	Psoriasis*
Chagas' disease*	Raynaud's disease*
Cohn's disease	Rheumatoid arthritis*
Depressive Psychosis*	Sarcoidosis
Discoid lupus erythematosis	Schizophrenia*
HIV infection*	Ulcerative colitis*

* anecdotal evidence for a beneficial effect already available.

Conclusion

The evidence from tuberculosis is becoming overwhelming that immunotherapy with an injection of killed mycobacterium vaccae can play an important part in treatment. It appears to act as a TH1 adjuvant, simultaneously down-regulating TH2 mechanism. Thus it should be useful in improving treatment for new cases and for chronic cases, though the later may require repeated injection for an optimal effect. Infection with drug resistant bacilli are likely to respond to immunotherapy just as well as do those with drug sensitive bacilli.

The potential value of this apparantly very safe treatment in non-mycobacterial diseases is only just being explored. Early information suggests that it may have a major impact, even in such diseases as cancer, atherosclerosis and the major psychoses.

The Enigma Of The *'Persister'* Form Of The Tubercle Bacillus

John M Grange

One of the best known of the many dogmas in the discipline of tuberculosis is that the disease is a biphasic one. Thus it is generally taught that initial infection causes a primary lesion which progresses to clinically overt disease within three years of infection in about 5 percent of cases. In the other 95 per cent , the focus of infection becomes dormant and usually stays so for the remainder of the person's life. In about 5 per cent of cases, however, the dormant foci reactivate leading to post-primary tuberculosis.This reactivation may occur years or decades after the initial infection.

This natural history of the disease led to the concept of a dormant or semi-dormant 'persister' form of the tubercle bacillus. This concept, and the supposed nature of the persisting bacilli, has drawn strength from two further dogmas, both of which have been seriously questioned.

The first dogma is that all post-primary tuberculosis is the result of endogenous reactivation of latent infection and that exogenous reinfection never, or very rarely, occurs. If this is the case , it would certainly demand the existence of a persisting form of the tubercle bacillus. It is, However, now known from a number of epidemiological studies, assisted by the recent application of DNA 'fingerprinting' techniques, that exogenous reinfection is a much more common occurence than previously suspected.[1]

The second dogma is that the tubercle bacillus is able to exist in various forms that are quite unlike the classical acid-fast bacillus. The reason that this idea developed was that early workers were puzzled by the fact that they were unable to demonstrate acid-fast bacilli microscopically in all lesions of human or cattle that were obviously tuberculosis in origin .This seemed to imply that the ba

cilli could exist in some form not easily demonstrable by conventional microscopy. Hans Much, for example, noted that such apparently abacillary tuberculosis lesions in cattle often contained numerous small densely staining granules which he regarded as being a form of the tubercle bacillus.[2] Other workers did not accept this claim and postulated that the granules were artefacts, such as mitochondria and other debris from necrotic cells. Also, Much's concept was not helped by his subsequent claims that the granules could be isolated from cabbages and other sites unlikely to contain tubercle bacilli.[3] The main reason why it is no longer necessary to postulate the existence of an aberrant form of mycobacteria is that it has been clearly demonstrated that relatively large number of acid-fast bacilli may be present in a specimen yet may be easily missed on microscopical examination. Thus, for example, sputum must contain at least 5,000 acid fast bacilli for them to be detectable on a standard smear examination.[4]

Nevertheless, the idea of the existence of unusual forms of mycobacteria, such as cell-wall free forms of minute, filter-passing forms has never entirely been refuted. From time to time there have been tantalising reports in the literature of the isolation of such forms from clinical material of their experimental production. [3,5-7] Several workers have indeed claimed that such forms are responsible for chronic granulomatous diseases such as sarcoidosis and Crohn's disease - claims supported in some cases by the apparent presence of specific mycobacterial nucleic acid, detectable by the polymerase chain reaction, in lesions of these diseases.[8]

Despite the objections to the original concepts that strongly pointed to the existence of latent, persisting forms of tubercle bacilli, there can be no doubt that some form of latency occurs. Thus, for example, in the United Kingdom, cases of tuberculosis due to the bovine tubercle bacillus - *M. bovis* - still occur in elderly persons, even though the source of infection was virtually eradicated in 1960. Also , the same molecular approaches that have clearly demonstrated exogenous reinfection have also confirmed the occurrence of endogenous reactivation.[1]

Given, therefore, that bacillary latency exists, it is necessary to consider what form or forms the latent bacilli take. One hypothesis is that the anoxic conditions within the lesions induce metabolic changes enabling the bacilli to enter a truly dormant state.[9] This

hypothesis has some backing from *in vitro* work but it is not clear to what extent such experimental systems reflect the *in vitro* situation. The problem with this hypothesis is that it is well known that conditions leading to immunosuppression strongly favour the reactivation of disease. It is difficult to explain how the immune system could affect the state of a completely dormant organism within dense, anoxic scar tissue. Another problem is the apparent efficacy of isoniazid in preventing infected but healthy persons from developing overt reactivation tuberculosis. The paradox, for which no rational explanation has ever been given , is that isoniazid , among all the antituberculosis agents, is most dependent on bacillary replication for its activity . Thus, based on the general concepts of bacterial dormancy, isoniazid would appear to be the least suitable agent for sterilization of inactive tuberculous lesions.

An alternative hypothesis is that the bacilli are, in fact , replicating (possibly slowly) but are being removed by the host's immune defences at a roughly similar rate. This would explain the weakening of the immune defences as a cause of reactivation. It would also explain the efficacy of isoniazid in preventive therapy.

Another form of dormancy is that which occurs during chemotherapy of clinically overt tuberculosis. It is well known that during modern short-course chemotherapy there is a rapid killing of the freely-replicating extracellular bacilli in the cavity walls during the first week or two (the early bactericidal effect) but that treatment must be continued for six months during which time dormant , or near dormant , organisms, probably mostly intracellular are killed at a much slower rate. Whether these 'persisters' are identical in nature to those present in the latent phase between primary and post-primary disease is not at all clear. Animal systems for the study of bacterial dormancy such as the 'Cornell model' in which persisting organisms are generated by treatment with isoniazid and pyrazinamide may not be relevant to all forms of dormancy. [10] The Cornell model has been investigated by the application of nucleic acid amplification techniques and large amounts of mycobacterial DNA , much more than accounted for by the numbers of cultivable bacilli, has been detected.[11] While this DNA may represent unusual, non-cultivable variants of the tubercle bacillus, it may simply indicate the presence of dead bacilli, or free mycobacterial DNA , in the tissues. The important point is that bacilli persisting

during chemotherapy are not killed by isoniazid - the only reason for the inclusion of this agent in the continuation phase of therapy is to kill any rifampicin resistant bacilli that might commence replication .[12] This again raises doubts that the 'persisters' in healthy infected persons and which are apparently inactivated by isoniazid monotherapy differ from bacilli that persist in a viable state during chemotherapy.

One of the problems faced in considering bacterial persistence is the term ' infection ' which , in fact, is an extremely vague one, with subtly different meanings to the microbiologist, immunologist, epidemiologist and physician. We are, for example, repeatedly reminded by the World Health Organization that one third of the World's population is infected by the tubercle bacillus and that it is from this infected pool that the 10 million or so new cases of tuberculosis arise each year. We cannot be sure, however , that all of these 'infected' people- which for practical purposes means those who are tuberculin positive - actually harbour viable tubercle bacilli for the rest of their lives. The fact that persons who are 'dually infected' by *M. tuberculosis* and the HIV have only about a 50 per cent chance of developing overt tuberculosis (and some of this may be due to reinfection) suggest that not all 'infected' persons actually harbour viable tubercle bacilli.

Thus one possibility is that, following initial replication of bacilli and the formation of the primary complex, the immune responses (in all except the minority of patients who develop potentially fatal forms of primary disease such as miliary or meningeal tuberculosis) cause a continuous decline in the number of bacilli, somewhat analogous to radioactive decay, so that a time may eventually be reached when all bacilli are destroyed . Alternatively, the bacilli may establish some form of symbiotic relationship with the host so that they persist, possibly with a slight rise and fall, for the remainder of the person's life.

It might appear that interest in the dynamics of persisting tubercle bacilli is a purely academic one, but in fact it bears great relevance to vaccination strategies. If a proportion of the human population is able to mount a sterilising immunity, rather than a response that merely induces a long-term state latency, then it may well prove possible to induce this state in all of ,or atleast in the great majority of the population . This point needs to be carefully

considered in future vaccination and immunotherapeutic strategies.

In conclusion, the nature of the mycobacterial 'persister', without which we would have neither endogenous reactivation of tuberculosis or the need for prolonged courses of therapy, continues to be an enigma. Even the application of the sophisticated techniques of molecular biology has failed to shed light on the nature of these strange organisms . If , however, we could convincingly isolate a persister and determine its characteristics, we could take a great stride forward in understanding the dynamics of infection by M.tuberculosis and the treatment of disease.

References

1. Stocker N. Tuberculosis in a changing world. Brit Med J 1994; 309: 1178-1179.

2. Much H Die nach Ziehl nicht darstellbaren Formen des Tuberkelbacillus. Berl Klin Wochenschr 1908; 45: 691-694.

3. Stanford JL . Much's granules revisited. Tubercle 1987; 68: 241-242.

4. Rouillon A, Perdrizet S, Parrot R. Transmission of tubercle bacilli : the effects of chemotherapy. Tubercle 1976; 57: 275-299.

5. Calmette A, Valtis J. Virulent filterable elements of the tubercle bacillus. Ann Med 1926; 19: 553-560.

6. Chandrasekar S, Ratnam S. Studies on cell-wall deficient non- acid fast variants of Mycobacterium tuberculosis. Tubercle Lung Dis 1992; 73: 273-279.

7. Grange JM. The mystery of the mycobacterial 'persister'. Tubercle Lung Dis 1992; 73: 249-251.

8. McFadden JJ, Fidler HM . Mycobacterial as possible causes of sarcoidosis and Crohn's disease. J Appl Bacteriol 1996; 81: In press.

9. Wayne LG. Dynamics of submerged growth of Mycobacterium tuberculosis under anaerobic and microaerophilic conditions. Amer Rev Resp Dis 1976; 114: 807-811.

10 McCune RM, Feldman FM , Lambert HP, McDermott W. Microbial persistence. 1. The capacity of tubercle bacilli to survive sterilization in mouse tissues. J Exp Med 1996; 123: 445-468.

11. de Wit D, Wooton M, Dhillon J, Mitchison DA. The bacterial DNA content of mouse organs in the Cornell model of dormant tuberculosis. Tubercle Lung Dis 1995; 76: 555-562

12 Mitchison DA Hypothesis : the action of antituberculosis drugs in short course chemotherapy. Tubercle 1985; 66: 219-225.

Newer Drugs For Tuberculosis

Pattisapu R. J. Gangadharam

In spite of substantial progress in chemotherapy of tuberculosis, the disease still remains as a major health problem in the world. More unfortunately, disease due to multiple drug resistant organisms is increasing world wide, making an urgent necessity to discover and develop newer drugs to control this disease. In this presentation, the various attempts will be discussed under the following categories: 1. Conventional screening of (a) plant and (b) synthetic drugs. The synthetic drugs are grouped as (i) Fluoroquinolines, (ii) Macrolides (iii) Nitroimidazoles (iv) Oxazolidones and (v) Polaxamer surfactants. 2. Re-discovery of some old drugs; these include (i) Clofazimine (ii) Paramomycin and (iii) Allicin; 3 Structural analogues and derivatives of existing drugs-isoniazid, rifampin and pyrazinamide. Of particular importance will be the rifampin analogues, which include rifapentine, rifabutin, CGP series, KRM 1648 and T 9. 4 Permeability barrier modifiers, which include (i) penicillinase inhibitors, (ii) ethambutol (iii) DMSO and (iv) Hamycin; and 5. Metabolic inhibitors which include (i) Gangamicin, (ii) Calmodulin antagonists and (iii) Mycolic acid synthesis inhibitors. The structures of these various drugs and the available knowledge on the in vitro, in vivo, intracellular and clinical aspects of the chemotherapeutic properties of these various potential compounds has been discussed in this presentation. Finally the problems facing us in developing them commercially, mainly due to the inertia of the pharmaceutical industry are also discussed.

The Human Dimension Of Tuberculosis Control

John M Grange

" Along the way of justice there is life"
-Proverbs of King Solomon : Chapter 12, verse. 28

At the heart of the whole subject of tuberculosis control there lies a paradox. Since the discovery of the causative organism, *Mycobacterium tuberculosis*, by Robert Koch in 1882, a huge amount of research has been directed towards this disease. Indeed, the British Medical Council began its existence as the Tuberculosis Research Council. Following the discovery of the various antituberculosis drugs , many distinguished workers such as Tripathy and Prabhakar in Madras , devoted much time and energy to the development of the best combinations of these agents and the design of the modern short-course regimens that have the *potential* (and it is important to stress the word *potential*) to cure up to 98 per cent of patients. Obviously, these regimens and the infrastructures required for their deployment incur costs to the community but the World Health Organization has clearly demonstrated that,in terms of productive years of human life saved, tuberculosis is among the most cost effective of all diseases to treat.[1] The paradox, therefore, is that in 1993 , 111 years after Koch's epoch-making discovery, the World Health Organization took the unprecedented step of declaring tuberculosis a global emergency.[2]

This declaration is clear proof that, despite all the advances in modern scientific medicine, our attempts to conquer, or even constrain, tuberculosis have failed. Not only have they failed, but through

misuse and non-use of the facilities at our disposal, we have perhaps missed a golden opportunity to take a significant step towards controlling this disease. It is in the nature of the human species to seek a scapegoat, and in the case of tuberculosis this is the HIV pandemic. The serious consequences of the 'cursed duet' of dual infection by the tubercle bacillus andHIV has been the subject of numerous gloomy predictions, yet only 8 percent of cases of tuberculosis world-wide are HIV related ; meaning that 92 per cent are not. In any case , HIV infection does not prevent the patient from being cured of tuberculosis, thereby removing a source of infection from the community. Also, we have to bear in mind that if tuberculosis had been adequately controlled, as it could have been , in that 'window' between the discovery of rifampicin and the onset of the HIV pandemic, we would not be seeing dual infection today. This is well summed up in the words of Sir John Crofton, a pioneer of tuberculosis chemotherapy:"It is a sad reflection on society's incompetence that, more than thirty years after the methods for cure and prevention were evolved, and before the advent of the HIV epidemic, there were already more patients with active tuberculosis in the world than there had been in the 1950 s ".[3]

Among the serious contemporary problems in tuberculosis control is that of drug resistance. Though the disastrous and extremely costly effects of the emergence of multi-drug resistance have been clearly revealed by the recent epidemics in New York, [4] the global community appears to stubbornly avoid acknowledging the gravity of this situation, except on paper.

The present day armamentarium against tuberculosis is composed of about twelve drugs. Three of these, rifampicin, isoniazid and pyrazinamide are effective and relatively non toxic : the others are either relatively ineffective, toxic and /or expensive . New drugs such as fluoroquinolones and macrolides are in this category (the author was prescribed a course of clarithromycin for a *Helicobacter pylori* stomach infection and abandoned this medication after one week owing to very unpleasant side effects). The question is whether additional drugs will become available certainly, no drug more effective than rifampicin has emerged during the 25 years or more since this agent was introduced. Also , there must surely be a very

finite number of agents, or classes of agents, that are lethally toxic to the tubercle bacillus yet leave human cells unscathed. What is extremely worrying is the short period of time, a mere two decades, over which multi-drug resistance has emerged.

Consequently, unless there is a global re-evaluation of the control of the available drugs , the nightmare scenario of untreatable tuberculosis could soon become reality. (Of course, it is to be hoped that immunotherapy of tuberculosis with heat-killed *Mycobacterium vaccae*, which at the time of writing is undergoing extensive clinical trials in South Africa, will play a major and highly beneficial role in the control of the disease. Unless this innovation proves successful and is rapidly deployed, the outlook is gloomy indeed.)

The present global toll of tuberculosis leads therefore to the regrettable but unavoidable conclusion that the current efforts to control tuberculosis are deplorably inadequate. Possibly for historical reasons, the subject of tuberculois is riddled with dogmas which so often prevail over imaginative, realistic and common-sense concepts.[5,6] It has been stated that conceptually excellent control programmes designed by international agencies are often totally unworkable in practice,[7] and one dedicated African worker has commented that, in many countries, control programmes only exist on paper.[8]

If we are to make an impact on tuberculosis world-wide, and we may well have only a limited period in which to do so , we need to look carefully , critically , undogmatically and philosophically at the root cause of the current problems and difficulties. In particular, we need to take a much more holistic view of the disease. This , however, from the standpoint of Western medicine , will be no easy task. There can be no doubt that Western 'scientific' medicine has contributed enormously to the relief of human suffering. Nevertheless, the reductionist and mechanistic principles on which it is founded render it virtually incapable of addressing many of the major health problems facing the planet today. At a medical colloquium in London, the Prince of Wales remarked that "The whole imposing edifice of modern medicine, for all its breathtaking success is, like the celebrated tower of Pisa, slightly off balance!"

In the Western world, we have become enchanted and beguiled by what might be termed the *molecular mythology* - the strange belief (and it is no more than an act of faith or, perhaps , even self deception) that the DNA of the tubercle bacillus is like some magic well which, if delved deep enough, will yield a panacea. It may well be that the path from molecule to man will lead to great and beneficial discoveries. So far, it would appear, it has not done so : we still have no vaccine other than the not very effective BCG , diagnostic tests continue to prove frustratingly non-specific and insensitive and 'designer drugs' remain a mere pipe dream.

Clearly, no discussion on global control of tuberculosis can avoid mention of finance. Drugs, diagnostic services and health care personnel must all be paid for. The sad fact is that the global community , which is only too ready to give lip service to the nightmarish problem of tuberculosis, becomes exceedingly cloth-eared when called upon to contribute to the cost of the necessary control measures.(In this context, the author is ashamed and embarrassed to report that the United Kingdom is too mean and miserly to pay its national membership fee to the International Union Against Tuberculosis and Lung Disease.)

Table 1.

Deaths in persons aged over 5 years from infectious and parasitic diseases and external aid given for their control.*

Disease	Deaths	External aid (US Dollars)
Tuberculosis	1,900,000	55,000,000
Diarrhoea	400,000	16,000,000
Malaria	300,000	47,000,000
AIDS	200,000	185,000,000
Parasitic disease	200,000	74,000,000
Leprosy	2,000	77,000,000

*** Data from World Health Organization.[2]**

Table 1 shows the amount of external aid given for control of the six major infectious diseases world-wide.[2]There is almost an inverse relation between the number of deaths due to the diseases and the funding provided, with tuberculosis showing a particularly

marked discrepancy. The World Health Organization has calculated that with the continued level of interest and support, the annual number of deaths from tuberculosis will rise to 4 million by the year 2004. With proper funding the annual number of deaths could be cut to 1.6 million.[2]

The sum required by the WHO for the achievement of this goal is 111 million US Dollars each year - $100 million for the actual programme and $11 million towards the management costs to ensure that the funds are well spent. This annual sum is the cost of a single jet fighter. In the document in which the WHO declared tuberculosis a global emergency, [2] the TB Programme Manager, Dr. Arata Kochi, wrote :

" Tuberculosis is one of the world's most neglected health crises . In spite of its alarming danger, surprisingly little action has been taken to address the TB epidemic.....Every day, I ask why this situation is allowed to continue. Is it possible that no one really cares whether 30 million people will die in the next decade from TB ? How can TB be such a neglected priority when it is one of the most cost-effective adult diseases to treat ?... Can we comprehend the magnitude of this injustice ?... The growing TB epidemic is no longer an emergency only for those who care about health,but for those who care about justice."

In this respect, an unavoidable issue in tuberculosis is that , in both developed and developing nations, tuberculosis is principally a disease of poverty and deprivation. The solution to this issue is largely a political one although it is not entirely outside the domain of the medical profession as physicians have always been powerful advocates of social reform and change. At the conference in London at which the WHO declared tuberculosis a global emergency, McAdam listed 'Ten Commitments' required for tuberculosis control.[9] Nine of these are concerned with the control programmes themselves but tenth relates to the fight against poverty, demanding".... a much wider commitment internationally to reduce the economic inequalities which allow tuberculosis to remain such a preventable scourge all over the world".

Of course, provision of financial aid by the international agencies such as the WHO , the UN and the Overseas Development

Organizations of the wealthier nations is just the beginning. Likewise, adoption of WHO - recommended control policies without very careful consideration of a whole host of local factors is a recipe for failure. In recent years, the TB Unit of the WHO has convened many meetings of leading tuberculosis workers in order to formulate control strategies but these can form the general, though undoubtedly firm foundation on which to build local programmes. It is quite remarkable how much time and effort can be wasted, and real harm done, by a failure to actually observe a programme in action and to audit its efficacy. The author once worked in a country where education, laboratory services and primary health care were directed by three separate government ministries. Consequently, poster would suddenly appear in a village urging people with chronic coughs to attend the local clinic for sputum microscopy, only to find that the clinic had no functional microscope or , even if it did, there were no available drugs with which to treat the patients.

Fortunately, the need to take local factors into account is increasingly recognized by donor agencies. For example , the International Development Research of Canada financed a study on 'Social and operational constraints in tuberculosis control in Maharashtra, India' and a seminar on Urban Tuberculosis Control which has been published.[10] This book is a mine of useful information and should be read by all those involved in this subject.

In many countries, health care is provided by government hospitals and clinics , in which knowledge and experience in managing tuberculosis may vary enormously in quality. In several countries, including India, non -governmental organizations (NGO) also play a key role. The Foundation for Research in Community Health has , for example , published a useful survey of the activities of a number of NGOs in Western India.[11] The NGOs have an important role to play in the education of tuberculosis physicians, whether in the public or private sectors, as well as nurses, paramedical workers and the local community.They should also ensure a regular stock of effective drugs for supply free of charge to the patient and the close supervision of such supplies. Hand in hand with supervision of therapy there must be a genuine caring support of the patient who

will doubtless have many problems and anxieties in addition to the disease.

These factors are essential for case finding as the population will only regularly attend clinics with a reputation for providing effective therapy in a caring and efficient manner. The author has experience of a city hospital where patients with pulmonary tuberculosis only presented when they had very advanced disease- three quarters of patients presented with haemoptysis - because they knew that the cost of curative therapy would be far beyond their means.

Continuing education of medical practitioners is essential for the provision of curative therapy in a cost-effective manner. A highly respected Indian physician , Dr. Mukund Uplekar, surveyed the prescribing habits of 100 practitioners in the private sector and found that they used 80 different drug regimens, many being considerably less effective and much more expensive than the standard WHO - recommended regimens .[12] Ideally , drugs should only be prescribed by accredited workers, who also understand the need for close supervision of therapy. Education programmes for paramedical workers provides a team of people of very great help in the supervision of therapy which the WHO now considers essential for the control of tuberculosis. The current WHO slogan is DOTS - Directly Observed Therapy, Short Course.

The concept of DOTS is fine in principle, and in New York where the authorities can afford a large team of health workers. In many regions, notably rural ones, serious problems are encountered, although these may be greatly alleviated by training workers, preferably those selected by the local community on account of their dedication and reliability rather than high educational qualifications. In Africa, a number of studies in different countries have revealed a close relation between the caring and kindness shown by such workers and the successful outcome of therapy.

Finally, education of the community as to the cause and infectious nature of tuberculosis and the principles and effectiveness of treatment is a way of enhancing case-finding and case-holding. In particular, it is important to attempt to remove the stigma attached to tuberculosis . In London, a physician who is recruiting tuberculosis patients to an immunotherapy study stated that he had

difficulties with patients from certain ethnic minority groups as they went into a state of deep shock and despair for several days when told the diagnosis.

Turning from the infrastructure to the individual, there are numerous factors to be considered. The word that appears again and again in the tuberculosis literature is *compliance*, also called *adherence*; in reference to completion of a course of therapy. So often, the blame for non-compliance is laid at the feet of the patient but , on careful analysis , the physician or the medical service is usually the culprit.

The point that cannot be overstressed is that compliance is directly related to the quality of medical services. [13,14] Many tuberculosis physicians have observed that the establishment of a good and trusting doctor-patient relationship is essential for success of treatment. [15] A British government publication, *The Health of the Nation* , states that "A key part of quality is how care is delivered - such as the provision of information or the attentiveness of friendliness of staff. The importance of the quality of service in terms of the patients' perception of health care experience should not be underestimated. The quality of service can have a therapeutic value and be of enormous psychological benefit".

If serious problems of doctor-patient communication occur in culturally relatively homogeneous populations, these are greatly magnified in societies where tuberculosis is common in ethnic minorities, as for example, in the United Kingdom. Not only are there linguistic barriers to be overcome, but the practitioner may unwittingly cause grave offence to patients and totally fail to understand their perception of disease. [16] An awreness of these problems led an Asian physician working in London to establish the discipline of ' transcultural medicine '.[17]

Another crucial element of good caring medicine is the availability of high quality drugs at no cost (or at most a nominal cost) to the patient. There are many horror stories of patients travelling large distances at high cost and discomfort only to be told on repeated occasions that the drugs have not arrived. Likewise there are parts of the world where patients are obliged to pay grossly inflated prices for drugs that are sub-optimal, time - expired or even counterfeit. [18]

It is now fully recognised that unregulated over-the-counter sale of antituberculosis drugs is a sure way of generating drug resistance, but it is unrealistic to expect covert dealing in these drugs to cease unless the state makes them readily available, though under supervision, to those who genuinely need them.

There is one final area of the human dimension of tuberculosis control that, in the author's opinion, is of the greatest importance yet is one that is particularly neglected. That is the spiritual dimension of medical care. Although in the Western world, religious leaders often have limited influence nowadays, this is not the case in many other parts of the world. The WHO has stressed the need for involvement of religious groups: " Religious leaders must use their stature to address inequities in health care for poor people. Since there is a cure for tuberculosis - a cure that is not being fully used - TB is no longer a medical epidemic, but an epidemic of injustice".[2] Some religious leaders in India , such as Sri Ganapati Sachchidananda Swamiji in Mysore, are doing an enormous amount of good work in establishing clinics, hospitals and eye camps, as well as schools and orphanages. But , by analogy with the situation in the United Kingdom, perhaps the medical profession is, in general, rather reluctant to forge a working relationship with ministers of religion. If so, that would be a great pity, as throughout the world, religion and medicine worked in close harmony until the advent of post-Cartesian 'scientific' medicine which separated the mind and soul from the body. In this context , there seems to be no reason what so ever why the fruits of modern scientific research cannot be harmoniously combined with whole person-centred medical philosophies such as Ayurveda.

Another reason for considering the spiritual dimention of medical care is that the mind-body separation so characteristic of Western medicine is, in fact, no longer tenable. There is now clear evidence that the immune response in tuberculosis, and the 'choice' between protective and tissue-damaging immune responses, is affected by the endocrine system , in particular by the balance of certain adrenal hormones; a balance which in turn, is effected by the pituitary, the hypothalamus and the higher centres of consciousness.[3] In addition, direct connections between the immune system

and the autonomic nervous system have been demonstrated. Thus , the relief of stress and fear may well make a great difference to the recovery of the patient,[20] and in this respect a minister of religion has much to offer. These concepts, now supported by modern scientific studies, should come as no surprise to those versed in the philosophy of Yoga.

In conclusion, this paper gives a brief and superficial synopsis of the numerous factors in the human dimension that require serious attention if the human race is ever to rid itself of tuberculosis. A close financial and technical cooperation between the industrially developed nations which have a mere 5 per cent of the global burden of tuberculosis and the poor nations which have 95 per cent of the burden is essential . In this respect, there is one great step that the wealthy nation could take. Mankind will soon be at the beginning of a new millennium and a number of grandiose and extravagant plans for celebrating this occasion have been prosposed in the United Kingdom and elsewhere. But what greater way could there be of celebrating the 2000th anniversary of the birth of a man who infuenced the whole world with his teachings of love, self sacrifice and justice than to cancel the burdensome interest rates payable by the poorer nations on loans from the wealthier ones. In this context , we should bear in mind the statement of the World Health Organization,

"Tuberculosis in no longer a medical epidemic, but an epidemic of injustice".

References

1.Murray CJ, Styblo K, Rouillon A. Tuberculosis in developing countries : burden, intervention and cost. Bull Int Union Tuberc Lung Dis 1990; 65 (1) : 6-24

2. World Health Organization. TB -A Global Emergency. Geneva : World Health Organization. 1994.

3. Crofton J Foreword. In Davies PDO (ed). Clinical Tuberculosis . London : Chapman and Hall. 1994.

4. Dooley SW , S imone M. The extent and management of drug-resistant tuberculosis : The American experience. In Davies PDO (ed). Clinical Tuberculosis . London : Chapman and Hall. 1994.pp 171-189.

5. Grange JM , Stanford JL. Dogma and innovation in the global control of tuberculosis J Roy Soc Med 1994; 87 :272-275

6. Yanez A. Restrictive factors in the application of a tuberculosis control programme. Bull Un Int Tuberc 1982 ; 57 : 252-257.

7. Chaulet P. But the emperor has no clothes on ! A critique on the report of the joint

IUAT/WHO study group Bull Un Int Tuberc 1983 ; 58 : 153 -156.
8. Salomao A. Most African countries only have paper TB programmes .Sidalerte 1994. 33 (Supplement - TB & HIV): 18.
9. McAdam KPWJ. Back to the future : 'the ten commitments'. InPorter JDH , McAdam KPWJ, eds: Tuberculosis: back to the future .Chichester : John Wiley. 1994. pp. 267 - 276.
10 Chakraborty AK, Rangan S, Uplekar M(eds) Urban tuberculosis control: problems and prospects. Bombay : The Foundation for Research in Community Health. 1995
11. Rangan S, Iyer A, Jhaveri S. Non governmental organisations in tuberculosis control in Western India. Bombay: The Foundation for Research in Community Health 1995
12 Uplekar MW, Shepard DS. Treatment of tuberculosis by private practitioners in India. Tubercle 1991; 72: 284-290.
13. Rubel AJ , Garro LC. Social and cultural factors in the successful control of tuberculosis .Publ Hlth Rep 1992; 107: 626-636.
14.Sumartojo E. When tuberculosis treatment fails. A social behavioural account of patient adherence. Amer Rev Respir Dis 1993; 147: 1311-1320.
15. Sbabaro J Patient compliance with preventive therapy. Operational consideration Bull Int Union Tuberc Lung Dis 1990/91 ; 66 Supplement: 37-39.
16. Grange JM , Festenstein F. The human dimension of tuberculosis control. Tubercle Lung Dis 1993; 74: 219-222.
17. Qureshi B. Transcultural medicine. Dealing with patients from different cultures. Dordrecht; Kluwer Academic Publishers. 1989.
18. Onyebujoh PC, Abdulmumini T, Robinson S, Rook GAW, Stanford JL. Immunotherapy with Mycobacterium vaccae as an addition to chemotherapy for the treatment of pulmonary tuberculosis under difficult condition in Africa. Respir Med 1995; 89: 199-207.
19. Baker R, Zumla A, Rook GAW. Tuberculosis , steroid metabolism and immunity . QJ Med 1996; 89: 387-394.
20. Lerner BH. Can stress cause disease? Revisiting the tuberculosis research to Thomas Holmes, 1949-196. Ann Int Med; 124: 673- 680.

Tuberculosis Programme At Cross Roads: Issues Towards Mid-Course Correction Within The System

A K Chakraborty

There is considerable interest of late in tuberculosis control activities, globally as well as in India. This is especially so, following the publication of the World Development Report 1993- Investing in Health. It is now believed that there are few interventions that are as cost-effective as tuberculosis treatment. In fact, this intervention compares favourably to other health interventions in common practice, such as measles immunisation and oral rehydration. The question of success in cost-effective delivery of services, according to the World Development Report however, revolves round a flexible and responsive health system, able to provide necessary prevention and curative care and, besides & especially, on the development of human resources (education & income growth).

Goal of TB Control

Any programme, to be effective, must have its objective and ultimate goal set. Otherwise, its outcome cannot be measured continuously or from time to time. For tuberculosis programme, let it be assumed for the moment, that its goal is to control or eradicate tuberculosis. There is a serious problem, however, in using 'control' as

the goal/objective, since it is to be defined at first, if one were to judge the performance of the programme against this. Old text books had arbitrarily defined 'control', as achievement of 1% infection prevalence among the 14 years old children. This was just an off-the-cuff pronouncement without a scientific data base. Needless to say, it does not stand a serious scrutiny today.

Alternatively, based on experience in some of the Northern European countries, a data base is today available and a time table is set for these countries to achieve certain stages in TB control. [2] These are defined in Table 1, and the prospective time table for these countries is given in Table 2. However, as far as our country is concerned, the situation recommended in the definition appears to be a far cry!

It can be seen from Table 3, that for a hypothetical district population of 1.5 million, there could be some 750 smear positive new TB cases in a year (@500 new smear pos.cases per million, corresponding to1% ARI)[3]. For achieving close to elimination status, this incidence has to come down 1 to 2 occurrences. Up against this, it is needless to say, that one should be pragmatic with regard to setting the programme objectives/Goals for this country. Use of facile words like 'Control' or 'Eradication' without scientific basis, does not make the programme any better. This, on the other hand, encourages a kind of loss of accountability and permits laissez faire, being contrary to the principles and pursuit of the science of epidemiology.

One could, however, use operational definitions with regard to case finding and treatment activities under the District Tuberculosis Programme (DTP). Enough data is available to set the quantifiable objectives for health services, which could be measured as efficiency indicators. Of course, mathematical models are available to show that the programme activities, performed with these levels of efficiency, performed over a period, could result in reduction of the pool of infectors and new incidence of cases in the community.[4]

Before these quantified objectives are discussed, a few words could be said about the DTP, which was formulated in 1962. The DTP, as is well understood now, does not function optimally at present. The broad reasons, why it had to be so, are known. However, let it be unambiguously said, that the principles of the DTP had laid down, for the first time, the basis for a cost - effective and demand

responsiveTB programme. These cost-effective intervention principles have stood the test of time, and are enumerated in Table.

When the DTP functions with the above principles in operation, a certain dynamics is created in the activities of the DTP i.e., Case finding (CF) and Treatment (T). Based on the dynamics, the 'Potential' could be derived . (When the DTP works as laid down in manuals the resultant efficiency is called 'Potential', as different for 'Efficiency', i.e. when the DTP functions on an average, subject to actual operation of the system). The case load in the community, the potential and the efficiency of the programme operations are explained in the model given in Table 5, in quantifiable (therefore measurable) terms.

Operational Indices of Cost effective Programme

CASE FINDING (C.F.): It could be seen in Table 5, that for an average Indian district of 1.9 million population, there could be 6460 smear and culture positive patients of TB at any point of time ('prevalence cases'): 2584 of them smear positive, (prevalence of smear positive). Of these, 3230 cases (50% self-reporting) would present themselves for diagnosis on their own. [5] But only 2584 would be amenable for smear positive diagnosis (80% of culture positive cases detected on one smear). If it is hypothetised that 1/3 of the prev. cases could be newly added every year, there could be 879 new incidence smear positive cases. Thus, more number than smear pos. incidence cases could be found under DTP, performing as per potential.

As against this case finding potential (CFP), an average DTP is diagnosing no more than 950 cases (Case Finding Efficiency; CFE: 36%)[6]. It therefore stands reasonable to hypothesise that case finding (smear positive case), practised under the DTP, is not falling short of the number of newly occurring cases annually. It is another matter that a large No.of X-ray diagnosed case complicate the picture, there being 4 such cases for every smear positive case diagnosed. This remains to be taken care of.

TREATMENT (T) : Let the efficiency of treatment (TE) be set against this. At the observed TE under SR(50%) and SCC (79%) respectively, only 465 or 735 cases are converted into sputum negative. As the over all efficiency of the DTP (DTPE), as a system, it amounts to

only 18% and 28% respectively, calculated out of the potential of 2584 smear pos. cases (the number which could have been diagnosed and rendered negative with 100% CFE & TE). When the annual incidence of 936 smear pos. cases is considered as the problem, we cannot say that the performance is very effective, and the failure of DTPE is attributed more to be a function of treatment activity than anything else. Over a long term, the community could develop a large backlog of inefficiently treated patients with the resultant drug resistance among them, apart from the problem remaining as an endemic one over a century or more. How dismal the picture could get, should be evident when the WHO>N.T.I. - Pilot studies on treatment Cohort Analysis in Hassan and Mysore districts are considered : TE for SR - 12.5%, & 17.5% at DTC and PHI in Mysore respectively; the same being 37.5% and 25% in Hassan. For S.C.C., the results were only a little better at between 45 and 47% for the two districts. [7]

Incidentally, one more point needs to be underlined here, which presents serious lacuna in efficiency-measurement of the system. The DTP is supposed to have its own system of information generation through cross index cards, Book of Treatment, etc. The pilot study on monitoring, just referred to, showed that Cross Index cards & Master Book of Treatment cards were mostly absent in the districts. Repeat sputum exam was done in less than 20% of cases. Under the circumstances, cohort analysis became a perfunctory exercise.

Review of NTP & Mid-Course Correction Through RNTP

On going monitoring of the National TB Programme (NTP) as well as a review conducted by the ICMR, in 1992, had identified certain constraints in the performance of the programme, many of which administrative in nature. (Table 6)[8]. These got reflected in the inability of the NTP to achieve the desired cure rate of over 80% , among the cases diagnosed. Lack of drug supply, inadequate supervision of the services and a treatment adherence of below 30% had resulted in a cure rate of between 15-20% only. Over the years, this treatment dynamics had resulted in a situation under which tuberculosis was turning into an epidemic of "left over illnesses" of sorts, wherein incidence of new cases was only a third of total prevalent cases in the community (majority diagnosed earlier but not cured

due to inadequate treatment). Further, with the population having nearly doubled in course of the last thirty years or so since the NTP was launched, the existing DTP setup needed to be strengthened in order to deal with the problem in hand.

Lastly, regimens with the newer bactericidal drugs are now widely recongised as a potent tool in tuberculosis control, provided it is used effectively to bring about a cure rate of more than 85%. This can be seen to be achieved only through supervised administration of the drugs, at least in the intensive phase.

Under the revised programme (RNTP), some of the aforesaid issues have been addressed [9]. The objectives in short are to achieve a cure rate of 85% and above, reduce case fatality rate substantially (from nearly 15% to below 5%) and finally to increase the intervention-specificity (by reducing proportion of radiologically positive patients who are not bacteriologically confirmed amongst cases diagnosed). (Table 7). It is to be understood, that operationally, the programme remains essentially the same, that is, the services are rendered by the general health setup. NTP was essentially conceived as a part of the general health services. Under the proposed revised pattern also it remains so, both in principle and practice.

However, in the process of revision, the in-puts (drugs, supervisory personnel, microscopes and reagents) are proposed to be augmented. (Table 8) Supervision, which was one of the main constraints under the NTP, is sought to be made effective by organising a Tuberculosis Unit for a population of 500,000. It is proposed to make innovative use of the peripheral health workers to achieve supervised drug administration to each patient, atleast during the initial intensive phase (Table 9 & 10). It can thus be seen that the revised programme is far from an attempt to polarize it away from the general health services. On the contrary, it is an effort to considerably strengthen the services by utilising the existing system and building appropriate supervisory tiers. Further, through the innovative use of repeated sputum smear examinations to establish the bacteriological diagnosis of pulm tuberculosis, it is proposed to significantly reduce X-ray diagnosis, thereby cutting both the load and cost on treatment services (Table 11).

The RNTP uses three short course regimens (Table 12) specific to the three major categories, made during diagnosis following sputum and X-ray tests. The treatment regimens have the unique feature of a mandatory phase of Directly Observed Treatment with SCC (DOTS) during the intensive phase. The total adoption of SCC as well as the supervised administration are unique for the TB programme in India. In the past, the high cost of short-course therapy precluded its implementation in many countries with scarce resources. However, the cost of short-course regimens has dropped significantly in recent years. Countries or organisations, who can estimate their long term needs and can purchase supplies in bulk, are able to buy the drugs at even lower prices. More importantly, recent economic analysis has indicated that short-course therapy is more cost effective than 12-18 months of outmoded treatment and indeed, as cost-effective as many other health interventions, including oral rehydration and measles immunisation as already pointed out [10]. For example, analysis of the 8 month regimen used in programmes in Malawi, Mozambique and the United Republic of Tanzania has shown that treating smear-positive tuberculosis costs US $ 20-57 per death averted.

Anti-tuberculosis drugs are a major component of the incremental cost of treating patients with tuberculosis. Other costs, however, such as those related to programme management, hospitalization and diagnosis may be equally large. Costs of drug regimens, however, should not be compared in isolation from the cure rate achieved with the regimens under the field conditions. In some cases, more expensive regimens can be more cost-effective, because higher cure rates can be realised in practice.

The revised programme is being test run in India in some urban conglomerations of Bombay, Calcutta, Delhi and in the rural area of Gujarat. It is seen from the recently published initial reports that the results of treatment are highly satisfactory (93-100% sputum conversion)[9]. So, judged by this objective alone, the pilot phase of the revised NTP could be seen as gradually acquiring the prospect of being implemented as a full-fledged programme in wider areas of India on a staggered basis.

Table - 1 GLOBAL TUBERCULOSIS SITUATION -1

ERADICATION (E)

↑

ELIMINATION (EL)

↑

VIRTUALLY IDENTICAL WITH EL

Incidence of Smear Positive Cases:
Below 1 per 10 Million
(Prevalence of Infection:
General Population 0.1%)

↑

CLOSE TO EL

Incidence of Smear Positive Cases:
1 per Million Population
(Prevalence of Infection below 1%)

Source : STYBLO [2]

Table -2 GLOBAL TUBERCULOSIS SITUATION - 2

Group I

Netherlands, etc.

Prevalence of Infection

15% (Present)

(Incidence of Smear Positive Cases:
12-15 per Million)

|

9% (2000 A.D.)

|

1% (2025 A.D.)

(Incidence of Smear Positive Cases:
<1 per Million

|

0.1% (2050 A.D.)

Source : STYBLO [2]

Table - 3 GLOBAL TUBERCULOSIS SITUATION- INCIDENCE OF SMEAR POSITIVE CASES IN THE COMMUNITY

	Present	EL
Group III Countries	500/Million (750)	1 per Million (1-2)
Group I Countries	12-15/Million	1 per Million

( ) Number of average Indian district of 1.5 million population

Source : CHAKRABORTY [3]

Table - 4 COST EFFECTIVE INTERVENTIONS (Beyond Controversy)

* Infectious cases priority - other cases lower priority (except miliary/meningeal/seriously ill)
[Diagnosis certain - spread of infection to 12 per year till death in 2 years- Most suffering Group]

* Self Reporting cases be attended to
[Substantially large proportion of cases in Community seek relief (50 %) - They suffer the most - easy to diagnose by Microscopy] .

* Smear microscopy to be primary tool of diagnosis
[Transmitters are identified - smear pos cases suffer more, diag available readily - services can be made available wide spread - simple & reliable tool].

* Hospitalised Treatment not a must. Domiciliary treatment with a potent regimen adequate.
[However this should not be taken to a point of dogma - treatment regularity & adequacy to be ensured].

Table -5 FLOW CHART

Model on DTP efficiency as at present Vs. WHO recommendation

AVERAGE INDIAN DISTRICT:

Population 1.9 million --> (a)

Prevalence of cases --> (b)
6460

Cases presenting for diagnosis (50% of b) --> (c)
3230

Case-finding potential (CFP) (80% of c) --> (d)
2584

CASE LOAD -ALTERNATIVE HYPOTHESIS

b1= prevalence of smear pos. cases = 2584

b2= incidence of all cases = 2196

b3= incidence of smear pos.cases = 879

Case-finding treatment

INTERVENTION OUTCOME					
A		B	C		
Case-finding Level	No	Treatment Efficiency (TE)#	DTPE @ No	%	@@
At current case finding level (CFE $.36%)	936	Level TE 1	465	18.00	E1
	936	Level TE2	735	28.44	E2
At case finding level recommended by WHO (WRCF*-70%)	1809	Level TE 3	1537	59.50	E3

HYPOTHESES USED

(a) Population of an average Indian District = 1.9 million

(b) Prevalence of all cases 5+ age = 4/1000 population

b1) Prevalence of smear positive cases = 40% of b

b2) Incidence of all cases = 34% of b

b3) Incidence of smear positive cases =34% of b1

(c) Cases presenting at health institutions for diagnosis =50% of b

(d) Sensitivity of diagnostic tool at health institutions = 80%

LEGENDS

Case : Bacteriologically positive patient of tuberculosis.

CFP: Case finding Potential - proportion of cases diagnosed among those presenting themselves at health institutions (80% of c or 40% of b) = (d)

CFE $: Case-Finding Efficiency = $\frac{\text{performance}}{\text{CFP}} \times 100$

*WRCF: WHO recommended case-finding level 70% of b1 (2584)

#TE: Treatment Efficiency-sputum negativity achieved in cases found

TE.1: Cases on SR-compliance 45% at level 4; TE-50%

TE.2: Cases on SCC-compliance 56% at level 4; TE-79%

TE.3: TE=85% as recommended by WHO.(Compliance level & Regimens not stated)

@ DTPE: DTP Efficiency Sputum negativity achieved out of cases presenting themselves for diagnosis i.e., CFP(d)

@@ E1-E3: DTP Efficiency under various options of case holding including WRCT

Table - 6 NTP - INDIA : REVIEW - 1992

* Inadequate budgetary outlays & shortage of drugs.
* Undue emphasis on X-rays
* Poor quality sputum microscopy
* Emphasis on case detection rather than cure
* Poor organizational set-up and support for TB
* Lack of consensus regarding treatment regimens

Source : Sarin & Dey [9]

Table - 7 REVISED NTP : OBJECTIVES

Overall

To Interrupt the chain of transmission
To Reduce mortality & morbidity from TB

Operational

To cure at least 85% of all newly detected cases of pulmonary tuberculosis through supervised SCC

To detect atleast 70% of estimated incidence of smear positive pulmonary TB cases . Efforts at case detection after achieving required cure rate

Source : Sarin & Dey [9]

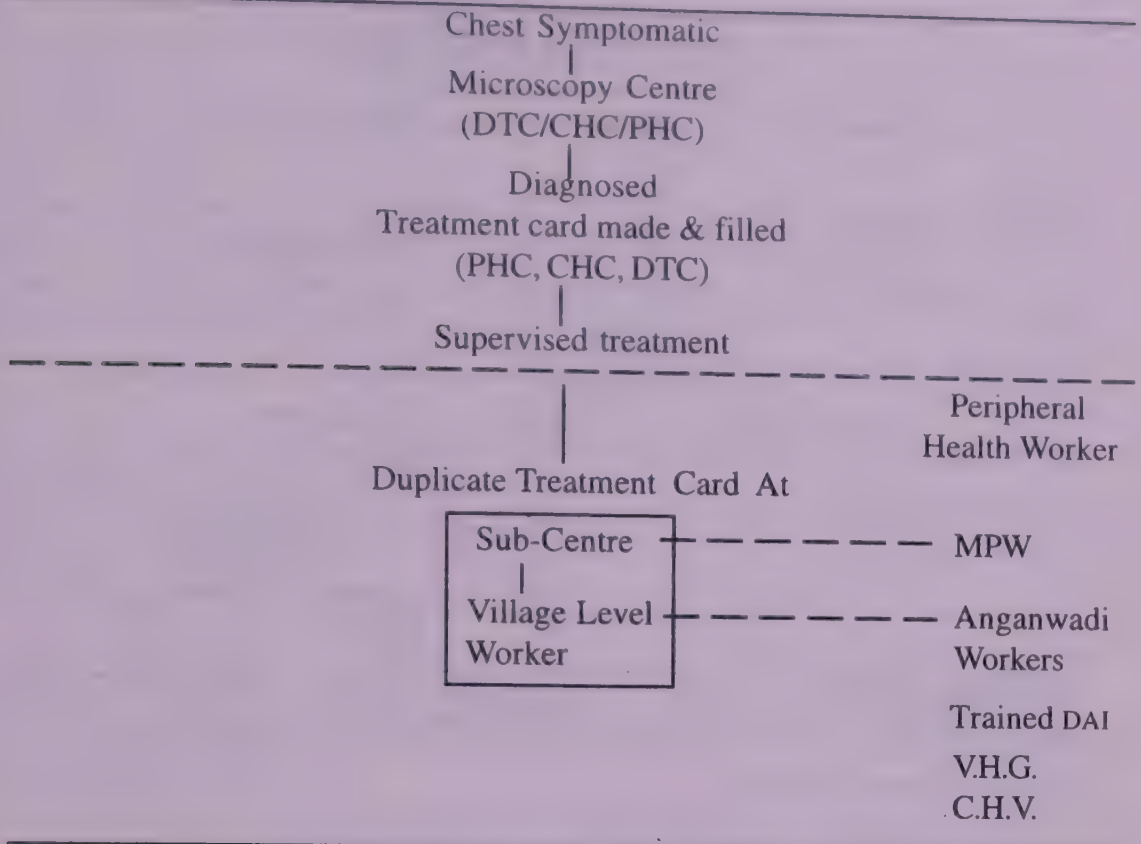
Table - 8 REVISED - NTP - STRATEGY : MANAGEMENT

* Uninterrupted Drug supply - blister combi- packs to all peripheral areas
* Strengthen DTC & state TB Centre
  - Effective implementation
  - Monitoring
  - Evaluation by cohort analysis
* Strength supervision beyond district level

Soruce : Sarin & Dey [9]

Table - 9 ORGANISATIONAL ARRANGEMENTS : RNTP

a) **General Model**

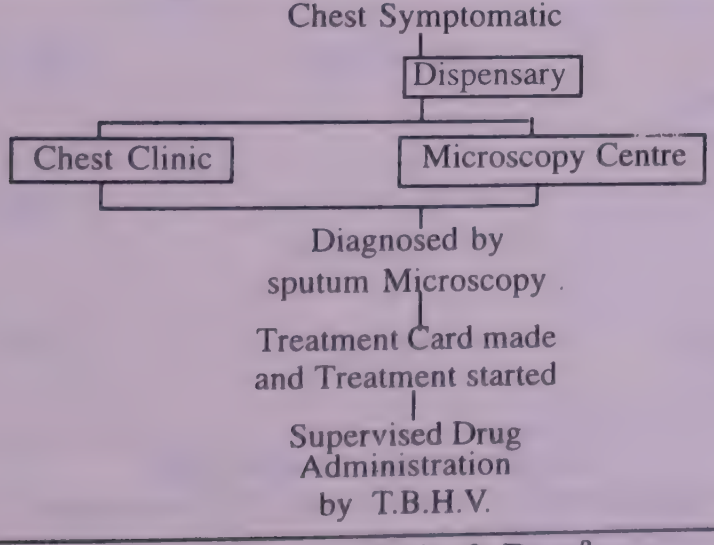


Source : Sarin & Dey [9]

Table - 10 ORGANISATIONAL ARRANGEMENTS : RNTP

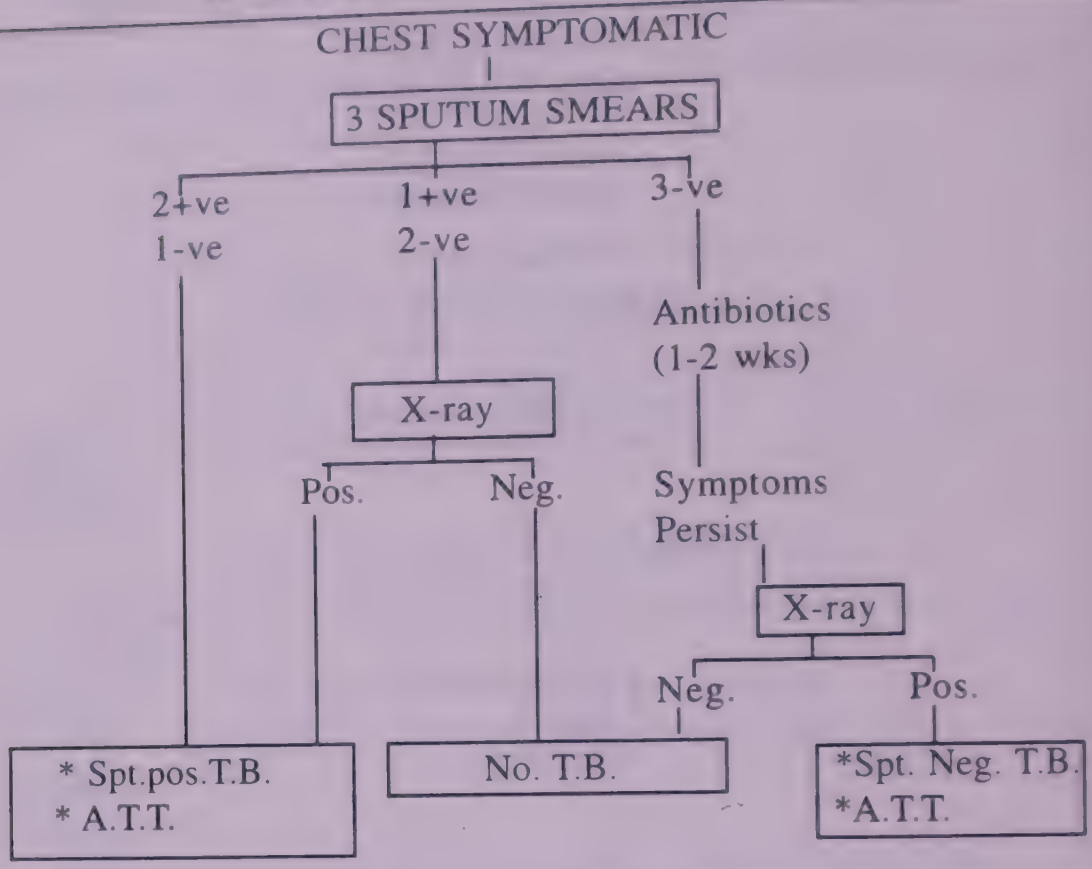
b) **Special Model**

(Where Primary health care not in position)



Source : Sarin & Dey [9]

Table - 11 DIAGNOSIS & MANAGEMENT



Source : Sarin & Dey [9]

TABLE-12 ANTI-TB DRUGS UNDER REVISED NTP

REGIMEN	INTENSIVE PHASE	CONTINUATION PHASE
CAT.I Infectious & seriously ill cases	EHRZ x 2 mth	HR x 4 mth
CAT.II Relapse & Failure cases	SEHRZ x 2 mth EHRZ x 1 mth	EHR x 5 mth
CAT.III Non-infectious & extra pulmonary	HRZ x 2 mth	HR x 4 mth

All drugs are given 3 days a week.

S-Streptomycin; E-Ethambutol; Z-Pyrazinamide.

H-INH; R-Rifampicin; Source: Sarin & Dey [9]

References

1 World Bank: World Development Report :1993. Investing in health 1993. Oxford University Press, New York pp -1-329 (+i-xii)

2. Styblo K : The estimate of tuberculosis in the Netherlands. Bull Int Union Tuber Lung Dis 1990; 65: (23). 49.

3. Chakraborty AK : Tuberculosis situation in India : measuring it through time, Ind J Tuber 1993; 40: 215-225.

4. Balansangameshwara VH, Chakraborty AK, Choudhury K: A mathematical construct of epidemiological time trend in tuberculosis- fifty year study, Ind J.Tuber 1992; 39: 87-98.

5. Banerjee D, Anderson S : A sociological study of awareness of symptoms among persons with pulmonary tuberculosis, Bull WHO 1963; 29: 665.

6. NTI : Monitoring of NTP, Annual Report NTI 1994-95, NTI, Govt. of India, Bangalore pp 1-42.

7. NTI : Pilot study on cohort analysis in Mysore & Hassan districts of Karnataka 1994, Unpublished, NTI, Bangalore.

8. Govt. of India, Ministry of Health, Tuberculosis Section : Review of NTP in India, 1992, unpublished.

9. Sarin R, Dey LBS: Indian National Tuberculosis Programme, Revised strategy, Ind J.Tuber 1995; 42: 95.

10. Murray C, Styblo K, Rouillon A : Tuberculosis in Disease control priorities in developing countries, Ed : Jamison DT et al, 1993, Oxford Medical Publication, New York, pp 1-746 (+i-xvii).

Why Are We Still Using BCG?

J L Stanford and C A Stanford

Introduction

Calmette and Geurin developed their vaccine for tuberculosis according to the accepted teaching of their day. Following the path developed by Jenner more than 100 years earlier, to be successful a vaccine should be live, and as similar as possible to the pathogen it was to counteract, yet not cause disease itself. Thus they selected a strain of bovine origin, at that time thought to be of low pathogenicity for man according to the teachings of Koch, though known to cause tuberculosis in cattle. They then followed the principle of attenuating their strain by culturing it repeatedly on media on which it did not grow well and occasionally testing it for virulance in guinea pigs. Eventually their strain lost the ability to cause disease in these animals which are very susceptible to both human and bovine strain of tubercle bacilli. They were then ready to test their vaccine on children. Originally the vaccine was administered by mouth, though currently prepared BCG vaccines are given by intradermal inoculation.

Today the daughter strains from Calmette's and Guerin's original have been distributed round the world where a number of manufacturers prepare the live vaccine from bacilli grown under a variety of conditions. Different manufacturers have sought different properties in their products. Some cause a vigorous local reaction with a proportion of recipients developing local lymphadenitis which may go on to caseation requiring drainage. Others cause a small and limited local reaction, only a proportion of which ulcerate. All should produce a positive reaction to 2 t.u. of tuberculin PPD in more than 90% of recipients on testing 8-12 weeks after vaccination. Some manufacture

approve tuberculin reactions of large diameter and others accept small responses provided they are in a large proportion of recipients. Arguments continue over the correct approach.

Numerous trials have been carried out with various manufacturers products and with a great variety of results. Discussions of why there is this variation in results are often vituperative and rarely conclusive. Thus we have in BCG a vaccine occasionally capable of inducing excellent protective immunity from tuberculosis and at other times failing or even increasing susceptibility. Results of a selection of these trials in prevention of tuberculosis or leprosy are shown in Table 1. It is important to note that equally good, and bad, results are found for both diseases.

Table 1

	Protection from tuberculosis		leprosy
UK	80%	Uganda	80%
Alabama & Georgia	14%	Burma	18%
South India	0%*		

By protection is meant the percentage reduction in number of cases in the vaccinated group in comparision with a control, non-vaccinated group, measured 5-10 years after vaccination.
*The initially reported result; subsequently this has improved to about 15% protection.

Another important effect of BCG vaccination may be to reduce the number of deaths occuring during subsequent tuberculosis disease. Thus in a study in The Gambia, none out of 80 tuberculosis patients with scars of past BCG died during the treatment period, in comparision with about 20% of such patients without scars ($p<0.01$). There did not appear to be any significant difference in the severity of presentation in the two groups and no evidence could be found that those with scars were more affluent or in receipt of a better diet than those without.

BCG appears to work as a vaccine by inducing a protective immune response that can be continuously boosted through contact with environmental mycobacteria, and which lowers the threshold

of immunological recognition for such organisms (see Figure 1).

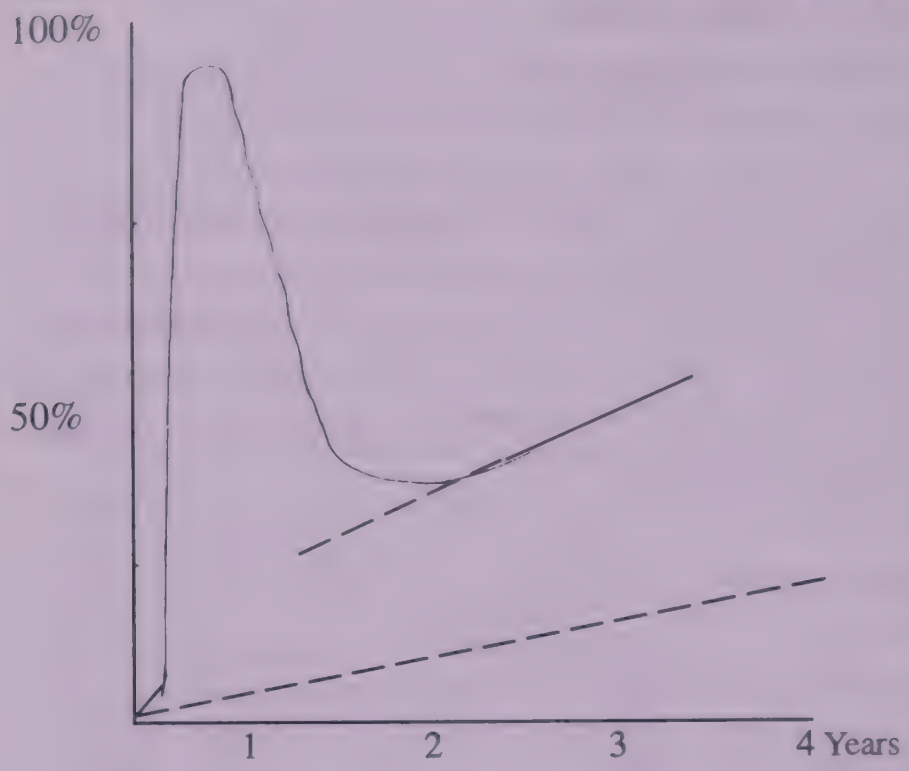


Figure 1

Diagram showing changes in tuberculin positivity with time following BCG vaccination in a country where contact with mycobacteria is infrequent. The rate of acquisition of tuberculin positivity in non-vaccinated individuals is shown as the lower broken line; it can be seen that BCG increases this rate by lowering the threshold of immune recognition of casually met mycobacteria

Thus when challenged with an infective dose of tubercle bacilli, a more rapid response is raised than in an unvaccinated person and in many cases this is sufficient to offset the development of disease. The vaccine also acts as a primary infection so that when tuberculosis does develop in a vaccinated person, it is of the more slowly progressing secondary, or adult type. Although BCG varies greatly in its ability to protect from secondary tuberculosis, whereever it has been used it reduces cases of primary disease such as miliary tuberculosis. It also restricts bloodborne spread of infection so that cases of tuberculosis meningitis and other types of extrapulmonary disease are fewer.

BCG fails to work in two situations : 1)where its effects have been pre-empted by earlier primary infection with tubercle bacilli or with environmental mycobacteria inducing a similar degree of protective immunity - this probably explains the failure in Alabama and Georgia. 2) It also fails to work and may even increase susceptibility to tuberculosis, in conditions where the abnormal immune status that accompanies tuberculosis already exists in the vaccinee. This occurs in those with latent infection with tubercle bacilli and in those meeting large numbers of slow-growing environmental mycobacteria. Thus in South India the failure was probably due to extensive daily contacts with strains of *M.avium* or *M.intracellulare*, and in Burma failure was probably due to excessive contact with *M.scrofulaceum.*

Despite its demonstrated merits, a vaccine made today based on the precepts behind BCG or a product of such variable quality producing such variable results would never reach the production line. Let us then discount BCG as the starting point for its successor and instead consider what we know now of tuberculosis and the nature of immunity to it, with a view to replacing BCG.

Mycobacteria their antigens and adjuvants

There are more than 50 species of mycobacteria now well defined and circumscribed and there are known to be many more species awaiting description in the environment. Anyone who cultures for the genus from soil will easily find strains that defy classification into known species. All these organisms have in common acid-fastness, cell-wall-associated adjuvant activity and a complex pattern of antigenic determinants, some of which are the mycobacterial versions of stress proteins sharing considerable aminoacid homology with those of man.

A great majority of mycobacterial species fall into one of two subgenera, generally called the slow-growers and the fast-growers. About equal numbers of species fall into each subgenes. In addition there are at least 2 species lacking the antigenic characteristics defining either of the subgenera and one of these is the leprosy bacillus. One of the most straightforward and simple ways of classifying mycobacterial antigens is immunodiffusion analysis, first introduced to bacteriology by Ouchterlony. Using sonicated

extracts of bacilli grown on antigen-free Sauton's medium, high titre antibodies can be raised in rabbits, and these sera can be used in double diffusion in gel against a range of bacterial extracts. By comparing adjacent reactions between the same serum and sonicates of different species, a pattern of antigenic relationships for the genus can be built up (Figure 2).

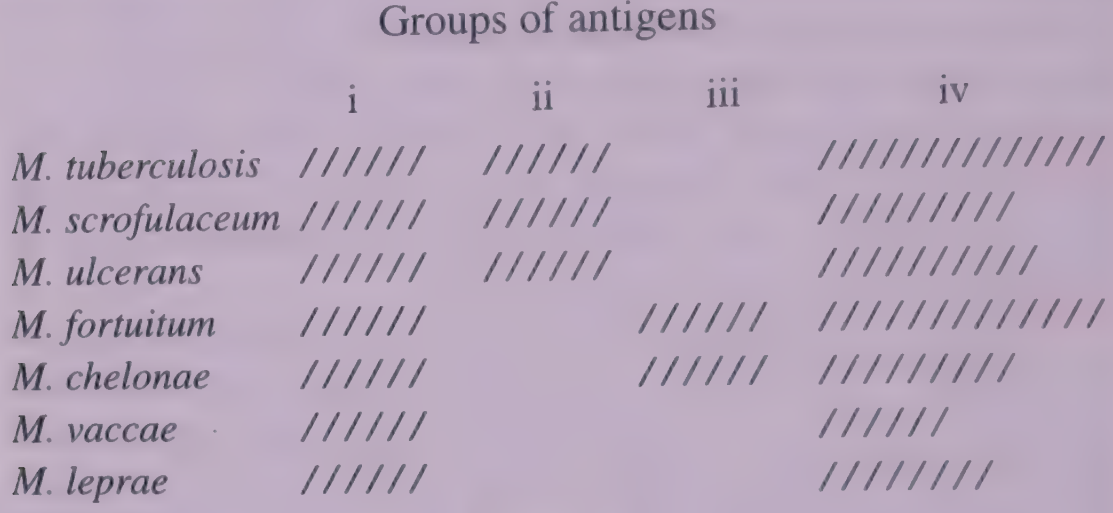


Figure 2

Diagram showing the antigens of mycobacteria demonstrable by double diffusion in sonicate preparations of various species using high-titre rabbit antisera. Representatives of typical slow-growing species are shown at the top, representatives of typical fast-growers are below them and the somewhat unusual species, M.vaccae and M.leprae are at the bottom. The common mycobacterial (group i) antigens shared by all species are shown on the left, the slow-grower associated (group ii) antigens are next to them, the fast-grower associated (group iii) antigens are shown next to them, and the species-specific (group iv)antigens limited to each individual species are shown on the far right.

The antigen can be clustered into 4 groups, group i, the common mycobacterial antigens are present in all species, and cell-mediated immune recognition of these antigens is induced by BCG in situations where it is successful. Antigens of group s ii and iii are of taxonomic improtance, but the part they play in protection or susceptibility from disease has been completely investigated. Group iv antigens are restricted to individual species and for pathogenic species immune reactions to them are associated with the development of immunopathology.

Additional evidence that the basis of immunity to mycobacterial disease is mediated by response to common mycobacterial antigens comes from the reduced ability of tuberculosis patients to respond to them. This is easily demonstrable by skin-testing patients and healthy control with reagents prepared from 4 different species, or by in vitro studies of lymphocyte proleferation in the presence of mycobacterial antigens.

Because both tuberculosis infection and BCG vaccination induce skin-test positivity to Tuberculin in most people, it has proved difficult to use Tuberculin in the investigation of interactions. In view of this we have studied BCG and alternatives in relation to leprosy as a model for how it may also work against tuberculosis. To do this we have used skin-tests with Leprosin A prepared from leprosy bacilli extracted from the tissues of experimentally infected nine-banded armadillos.

A study was set up in 1979 with the Mahatma Gandhi Memorial Leprosy Centre in Wardha, in which healthy persons living in leprosy epidemic villages were skin-tested with a range of tuberculins including Leprosin A. Nine years later it was found that 8 people had developed clinical leprosy and all were amongst those who had tested negative to Leprosin A 9 (see Table 2). Thus a positive reaction to Leprosin A appears to be a marker for immunity from developing clinical leprosy. We have subsequently used this test as a measure of potential success of vaccines in inducing protective immunity from the disease.

Table 2

The results of skin tests with Leprosin A carried out in healthy persons living in villages endemic for leprosy in 1979 . Within 9 years, 8 of those tested developed clinical leprosy. The Table also shows the distribution of these cases according to their earlier skin test results.

1979 results			
	Leprosin A +ve		Leprosin A -ve
	193/525		332/525
Developed leprosy in 1988			
	0/193	$p<0.05$	8/332

Table 3 shows that in situations where BCG appears to confer protection, positivity to Leprosin A is not directly induced, but conversion is enhanced after contact with leprosy bacilli in the environment. Thus after BCG vaccination early in life, children of leprosy patients steadly develop positivity with length of contact, and without developing disease.

Table 3

Positivity to Leprosin A by age and according to BCG scar or not, in healthy children living in Baba Baghi Leprosy Sanatorium, or in leprosy-endemic villages in Iran.

Age	BCG positive		BCGnegative
Leprosarium children			
1-7 years	11/25(44%)	p<0.002	0/18
8-12 years	34/40(85%)		1/2
13-20 years	25/27(93%)		0/1
Leprosy-endemic villages			
12-14 years	38/77(48%)	p<0.05	9/32(28%)

Besides antigens, mycobacteria contains a number of molecules with marked adjuvant activity in their cell walls. It seems that this adjuvant activity differs according to the mix of such molecules, or perhaps on small changes in their chemical structure. Thus different species may have a different spectrum of adjuvant activity and according to their presentation to the immune system different antigens may drive different responses. The cellular responses to common mycobacterial antigens appear to be protective and are lost in disease.

Attempts to improve on or replace BCG

What is wanted is a vaccine that always induces protective immunity in whichever country it is given, and which is equally effective against both tuberculosis and leprosy. This vaccine should not be live, should be simple and cheap to make, produce no disadvantageous generalised side effects and produce a local reaction no worse than that commonly produced by BCG. A number of fast-growing mycobacterial species were considered as candidates and a strain of Mycobacterium vaccae was selected. This strain had been isolated from the environment in Uganda where BCG had been shown to be effective against leprosy. At first this was used in combination

with live BCG, and the Leprosin A conversion achieved 1-3 years afterwards in regions of differing endemicity for leprosy are shown in Table 4. It can be seen that conversion after BCG alone varies from place to place, but the advantage shown by the addition of the M.vaccae increases as contact with M.leprae increases.

Table 4

Numbers and percentage of Leprosin A conversion after BCG alone, or with 10^7 killed M.vaccae added to it.

Endemicity for leprosy	BCG alone		BCG+M.vaccae
low or unknown	188/396(48%)		211/421(50%)
high	58/165 (35%)	p<0.05	74/158 (47%)
close contacts	99/176 (56%)	p<0.005	77/104 (74%)

Vaccination with 10^8 killed M.vaccae alone was used on Leprosin A negative close contacts with a BCG scar, or who were Tuberculin positive without a scar. Studies were carried out in South India and in Vietnam and vaccinees were retested with Leprosin A a year later. It can be seen from Table 5 that this too was followed by significant Leprosin A conversion in both countries.

Table 5

Reactions to skin tests with Leprosin A of mean diameter 3mm or greater one year after vaccination with 10^8 killed M.vaccae. Those vaccinated were either Tuberculin positive, or had a scar of previous BCG vaccination, and had responses to Leprosin A of zero or 2mm.

	Initial reaction*		One year later@
Vietnam	10/49(20%)	p<0.00001	34/49(69%)
South India	4/50(8%)	p<0.00001	31/50(62%)

*2mm reactions. @reactions of 3mm or more

Data from studies on childhood close contacts of leprosy patients in Iran show that both in combination with BCG, or given alone, M.vaccae improves on BCG by about 50% up to 10 years after vaccination. Formal vaccine trials have not been carried out, but it would

be reasonable to expect that both procedures would be improvements on BCG.

Evaluation of vaccines for tuberculosis and leprosy in experimental animals is largely pointless. First, almost any mycobacterial species can be used to produce a degree of protection from challenge with any other. Second, the short environmental experience of most laboratory animal species hardly equates with that of man, unless vaccination is given in infancy. Third, extensive maternal experience is likely to be conferred onto the human infants, transplacentally or in breast milk, modifying the effects of neonatal vaccination. Fourth, to be useful in man the vaccine needs to remain effective for many years, probably as a result of environmental boosting and this would be very hard to evaluate in animals, Fifth, animals have to be given an artificial infectious challenge to produce disease, usually with a dose considerably greater than most infectious doses that are likely to be met by man.

Despite these objections, experimental animals can be very valuable in investigating theoretical aspects of vaccination. Thus simple experiments in mice were used in the selection process for M.vaccae as a potential vaccine for use in man. At the time these experiments were carried out some 20 years ago, investigations in Uganda had suggested that M.vaccae was amongst the environmental organisms priming for a successful outcome to BCG vaccination and similar studies in Burma had incriminated contact with M.scrofulaceum as disadvantageous. In an attempt to demonstrate ugandan and burmese environmental influences, groups of mice were exposed to M.vaccae or M.scrofulaceum in their drinking water for a month. A month after completing this the mice were vaccinated with 10^5 BCG (Glaxo, freeze-dried) and their immune responses assessed by foot-pad testing with tuberculin. As shown in Figure 3, priming with M.vaccae enhances the response pattern found after BCG is given to non-primed animals, whereas priming with M.scrofulaceum results in a quite different pattern of responses. Subsequent challenge with a low-grade mouse pathogen, M.kansasii, showed improved protection in those primed with M.vaccae and increased immunopathology in those primed with M.scrofulaceum.

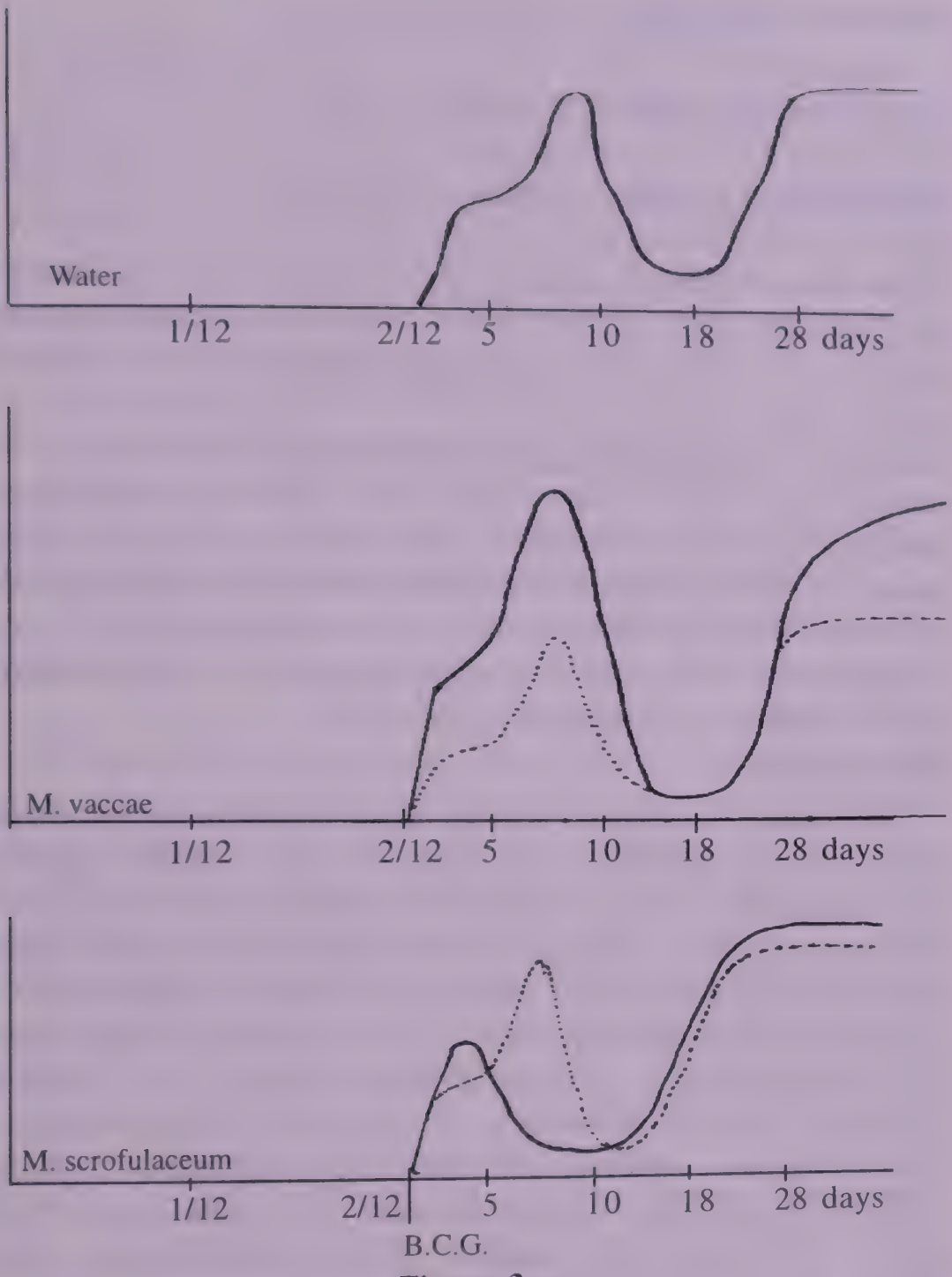


Figure 3

Mouse experiment showing the influences of exposure to different mycobacterial species on the immune consequence of subsequently administrated BCG vaccination. The top figure is what happens when no mycobacteria are added. Exposure to M.vaccae mirrors what happens to children in Uganda and exposure to M.scrofulaceum mirrors what happens to children in Burma. Note: Footpad responses to tuberculin at days 5, 10, 18 and 28 after BCG vaccination are plotted. The dotted line is the response of the control group shown for comparison.

Difference between BCG and M.vaccae

Whereas BCG is a live vaccine, M.vaccae is used killed. This would be disadvantage if killed BCG or tubercle bacilli were used because the amount of species-specific antigen in the 100-fold larger dose needed for a killed vaccine would be likely to cause severe local reactions and might well have other undesirable effects. This is not the case when non-pathogenic organisms such as M.vaccae are used, since their species-specific antigens do not evoke necrotic reactions. BCG contains the species-specific antigens of tubercle bacilli and in patients latently infected with tuberculosis, the additional antigen challenge might precipitate clinical disease. This dose not occur with M.vaccae which has marked immunotherapeutic effects in active tuberculosis and would be very likely to abort latent infection. Whereas BCG acts as an almost non-specific adjuvant for cell-mediated immunity, maximising whichever form predominates at the time it is administrated, M.vaccae promotes the Th1 pathway and down-regulates Th2.

Conclusions

BCG can be a valuable vaccine against both tuberculosis and leprosy, but its efficacy is very variable. This variability appears to be associated with advantageous or prejudicial priming from previous experience of environmental mycobacteria.This variable effect is associated with the non-specific adjuvant activity of BCG on cell-mediated immunity. BCG is not sacrosanct and there would be many advantages of using a killed vaccine if one could be developed. What is needed is a vaccine which would be independent of previous environmental effects, and capable of imposing TH1 associated anti-bacterial immunity in all situations. This is by no means impossible and the use of killed M.vaccae, either alone or added to live BCG offers one such route.

Mycobacterial Protein Antigens As Immunoprophylactic Agent Against Experimental Tuberculosis.

G.K.Khuller , Neelam Dhiman and Rakesh Sinha

ABSTRACT

Protein based immunoreactivities of cell wall and culture filtrates of Mycobacterium tuberculosis $H_{37}Ra$ were evaluated. Three proteins of Mr 71, 60 and 45 kDa were isolated by chemical treatment of mycobacterial cell wall and a panel of six different secretory proteins of apparent molecular mass of 15, 26, 30, 41,55 and 70 kDa were isolated from culture filtrate using column chromatography techniques. Amonst the isolated proteins, the 71 kDa cell wall protein and 30 kDa secretory protein were found to induce higher cell mediated (assayed by T-cell proliferation assay) responses in mice immunized with total cell wall protein (CW-Pr) and culture proteins emulsified in Freund's incomplete adjuvant (FIA) respectively. Further, immunization with pure 71 kDa/30 kDa emulsified in FIA resulted in enhanced T-cell responses which were skewed towards T-helper 1 (TH1) subset as determined by pronounced secretion of interleukin-2 (IL-2) and gamma-interferon (IFN-y). The protective efficacy of 71 kDa/30kDa protein was also significantly higher with reference to Bacillus Calmette Guerin (BCG) vaccine on the basis of increased survival and decreased bacterial load in different organs at 30 days post infection (p.in.) in mice challenged with LD50 of M.tuberculosis H37Rv. However, the degree of

immunity induced by both the antigens decreased with time (when challenged 8,12 and 16 weeks p.im.) but was still comparable to BCG. The mechanism of protection was also elucidated by adoptive transfer studies which showed that the protection was mediated by cooperative effect of CD4+ and CD8+T-lymphocytes by 71 kDa/30 kDa proteins. These findings suggest that the 71 kDa cell wall protein and 30 kDa secretory protein of M.tuberculosis are the key immunoprotective antigens and may be suitable candidates for development of alternative subunit vaccine against tuberculosis.

INTRODUCTION

Resistance to mycobacterial infection is mediated by a subpopulation of specifically activated T-lymphocytes against selected "Protective antigens" of mycobacteria[1]. Recent studies have shown that the protein antigens of mycobacteria, specifically those in avid association with the cell wall peptidoglycan, and those secreted into the growth media by actively growing cells, contain the immunoprotective epitopes that are recognised by the immune cells [2-6]. These proteins come in direct contact with the host immunoresponsive cells and are known to be recognised by T-lymphocytes at an early stage of infection, thereby, playing a protective role against the disease[7]. In recent years, several reports have appeared which indicate the role of purified protein antigens either used alone or in combination with other proteins, in inducing protection against tuberculosis [7-9]. However, more work is required to identify the immnoprotective antigens and evaluate their protective potential for the development of an antituberculous vaccine. Further, their mechanism of immunoprotection needs to be elucidated. Therefore, in the present study, we have screened the immunoreactivity of cell wall associated proteins and major secretory proteins in mice model to identify the immunodominant antigens. Further, their protective potential against experimental tuberculosis has been evaluated. The identified antigens could be used either alone or in combination for antituberculous vaccine development.

MATERIAL AND METHODS

Animals

Studies were performed with NMRI(inbred) strain mice, purchased from Central Research Institute, Kasauli (India). The

animals were kept in conventional housing condition and fed on mouse chow and water ad libitum.

Mycobacterial cultures

M.tuberculosis $H_{37}Ra$, M. tuberculosis $H_{37}Rv$ and M.bovis BCG were obtained from the National Collection of Type Cultures (NCTC), London and maintained on Lowenstein- Jenson's medium or modified Youman's medium under stationary conditions. M.tuberculosis $H_{37}Ra$ was subcultured in liquid Youman's medium and grown at 37°C on an orbital shaker.

Isolation of antigens

The cell wall assoicated and secretory proteins were isolated and purified as described previously[10,11].

Immunization of animals with purified proteins

The mice (in groups of 20 each) were immunized subcutaneously on day 0 and 7 followed by an intramuscular injection on day 14 with CW-Pr/71/kDa/total culture filtrate proteins / 30 kDa protein of M.tuberculosis $H_{37}Ra$ emulsified in FIA. Another group of mice were immunized with $1x10^6$ colony forming units (CFU) of BCG vaccine intravenously (i.v.) in 0.1 ml of saline by tail vein. Control animals were sham-immunized with saline in adjuvant.

Immune responses

At weekly intervals, 4-5 animals were exsanguinated and their spleens were removed aseptically.

Evaluation of cell-mediated immune (CMI) responses

T-cell proliferation assays were performed as described previously [7]. Briefly $2x10^5$ lymphocytes in a volume of 100 ul of RPMI- 1640 supplemented with $5x10^{-5}$M, 2-mercaptoethanol, penicillin-streptomycin (100U/mll) and 10% heat inactivated fetal calf serum. The cultures were incubated with or without antigen (100ul per well: 10 ug ml) at 37°C in humidified atmosphere of 5-10% CO_2 for 4 days. The cellular proliferation was investigated by pulsing cultures with 0.5 uCi of [^{3}H]-thymidine (BARC, India) and then incubated for 18 hrs. The cells were harvested on fibre glass filter, and incorporated radioactivity was counted on a liquid scintillation counter.

The culture supernatants from in vitro 71 kDa/30 kDa sensitized T-lymphocytes were harvested from parallel culture in a 24 well

plate after 48 hr of incubation for interleukin- 2(IL-2) and 96 hrs for gamma-interferon (IFN-y).

Cytokine assay

The amount of cytokine (IL-2 and IFN-y) present in culture supernatants were quantified by enzyme linked immunosorbent assay using commercially available kits (ELISA) from Genzyme Immunobiologicals.

Protective immunity to virulent challenge

After complete immunization (with 71-kDa-FIA/30kDa-FIA, BCG vaccinated and sham-immunized controls), the animals were challenged, 8,12 and 16 weeks p.im. intravenously with LD_{50} i.e. 3 x 10^7 CFUs of M.tuberculosis H_{37}Rv. Challenged animals were observed for 30 days for mortality and percent survival in each group was determined as an index of protective immunity. Enumeration of bacteria in the organs (spleen, liver and lung) of infected mice were done by plating serial tenfold dilution of organ homogenate on to the solidified Youman's medium supplemented with 1% (v/v) BSA and 2% agar powder. Colonies were enumerated after 5-6 weeks and were expressed as mean counts from three to five animals in each group.

Mechanism of immunoprotection

The 71 kDa/30 kDa immunized mice were sacrificed after 8 weeks and spleens were removed to obtain single cell suspensions. The spleenocytes were fractionated into CD3+, CD4+ and CD8+ subsets by negative affinity selection on T-cell enrichment columns obtained from R and D systems. The cells, at a concentration of $2x10^6$ cells/ml were intravenously (100ul) injected into irradiated recepient mice (500 rads of y-irradiation). Controls received CD3+cells from control mice. The animals were challenged with LD_{50} of M.tuberculosis H_{37}Rv after 2 days and survival rates were monitored till 30 days p.in.

Statistical analysis

Data of proliferative responses were analysed by student's t-test and protection data were evaluated using Chi square test.

RESULTS

Comparative immune response to purified proteins in animals immunized with total cell wall/secretory proteins.

The T-cell proliferative responses to three cell wall proteins and

six purified secretory proteins were compared upto the fourth week p.im. in mice immunized with 60 ug of total cell wall proteins /70 ug of total secretory proteins emulsified in FIA respectively.

The cellular response was monitored by T-cell proliferation assay in spleenocytes sensitized in vitro with all the pure antigens. All the cell wall and secretory antigens induced significant proliferation upto the fourth week p.im reaching the peak activity at the third week p.im as compared to sham immunized controls. However, the 71 kDa cell wall antigen [Fig 1(a)] and 30 kDa secretory antigen [Fig.1(b)] exhibited maximum T-cell sensitization throughout the observation period as compared to other antigens suggesting them to be immunodominant mycobacterial antigens. Silver stained SDS-PAGE profile of purified 71kDa/30 kDa protein are shown in Fig. 2.

Immunogenicity of purified 71 kDa/30 kDa protein

The immunoreactivity of 71 kDa cell wall protein (60 ug of 71 kDa FIA/mouse) and 30 kDa secretory protein (75, 37.5 and 18.75ug of 30 kDa FIA/mouse) in comparison to BCG vaccinated mice was studied by monitoring the T-cell response in invitro sensitized spleenocytes [Fig. 3(a) and (b)]. The response to purified antigens was higher than that obtained in total protein immunized mice as well as BCG vaccinated and exhibited an increase from first to third week p.im. Further, the response to 30 kDa was dose dependent with maximum proliferation observed with 75 ug of antigen in FIA (Fig.3c).

To characterize the T-cell response induced by immunization with 71 kDa/30 kDa and BCG immunization, supernatants from in vitro lymphocyte cultures were tested for the presence of cytokines viz IFN-y and IL-2 [Fig.4(a) and (b)]. No cytokines were detected in unstimulated cultures whereas, high levels of IL-2 and IFN-y were found in the culture supernatants of cells obtained from 71 kDa/30 kDa immunized mice as compared to BCG vaccinated.

Protective efficacy of 71 kDa/30kDa protein

The immunoprotective efficacy of the 71 kDa/30 kDa complexed in FIA and protection imparted by BCG vaccination in experimental animals was compared at 8,12 and 16 weeks p.im. The animals were immunized with 60ug of 71 kDa-FIA/ 75ug of 30 kDa-FIA

CONTROL

and $1x10^6$ CFUs of BCG vaccine. The 71 kDa immunized animals were challenged 8 and 16 weeks p.im. while 30 kDa-FIA immunized at 8 and 12 weeks p.im. The animals were observed for mortality upto 30 days p.in. The protection imparted by both the protein varied from 82.8-90% as compared to 65-70% in BCG vaccinated and 40-50% in sham immunized controls at 8 weeks p.im. (Table 1, Fig. 5). The protection was further substantiated by decreased bacterial load in the 71 kDa/30 kDa immunized mice as compared to BCG vaccinated and controls (Table 2).

The challenge period was increased from 8 weeks to 16 weeks for 71 kDa and 12 weeks for 30 kDa to eliminate the non-specific inflammatory responses to antigen and adjuvant. The protection to both 71 kDa and 30 kDa was found to decrease marginally on the basis of survival but was still higher than BCG (Table 3, Fig. 5).

Mechanism of immunoprotection

Adoptive transfer studies were performed to elucidate the mechanism of immunoprotection of 71 kDa/30 kDa. The recepient mice, passively transferred with CD4+and CD8+cells from 71 kDA/30 kDa immunized mice and challenged with LD_{50} of M.tuberculosis $H_{37}Rv$ exhibited greater surival (66-73%) which was further enhanced in case of total spleenocytes (86%) consisting of dendritic cells, Natural killer cells and macrophages in addition to CD4+and CD8+ T-lymphocytes (Table 4). The results suggested the cooperative effect of CD4+and CD8+cells in immunoprotection.

DISCUSSION

Mycobacterial cell wall associated and secreted proteins have been identified as potent inducers of immunity against tuberculosis [2-10], which is mediated by activation of T-cells and release of cytokines involved in acquired resistance [11-13]. The present study was undertaken to identify the immunodominant antigen of the mycobacterial cell wall, which come in direct contact with the immunoresponsive cells and the immunoprotective activity of purified secretory proteins which are responsible for early T-cell recognition.

The 71 kDa cell wall protein and 30 kDa secretory protein were found to be most immunoreactive exhibiting maximum T-cell sensitization (Fig.1). The 30 kDa protein was found to be glycosylated

with N-terminal amino acid sequence (APVDGVINTA) distinct from previously characterized Ag85 complex from M.tuberculosis H_{37}Rv and M.bovis BCG (MPT 39, MPT 44, MPT 45) [14]. The 71 kDa cell wall protein was found to be a non glycosylated protein with distinct ATPase activity (unpublished observation). Further, it differed from the extracellular 70 kDa on the basis of its non glycosylated nature and immunoreactivity.

Animals immunized with pure 71 kDa/30 kDa protein in FIA exhibited enhanced cellular responses which were higher than total cell wall protein/culture filtrate immunized or BCG vaccinated. This might be due to the presence of other antigens in total proteins which have lower immunoreactivity and might be influencing the immunoreactivity of pure protein or immunization with total protein/BCG does not provide effective immunization dose of pure protein. Further, significant IL-2 and IFN-y production was observed in 71 kDa/30 kDa activated T-lymphocytes (Fig.4) suggesting efficient activation of TH1 subset of CD4+T-lymphocytes which are important mediators of protective immunity [9]. These findings strongly suggest the potential of 71 kDa/30kDa protein for the development of antituberculous vaccine either alone or in combination. Our findings are in agreement with reports of other workers who have shown that protein antigens of mycobacteria activate the TH-1 type of T-cells which secrete IL-2 and IFN-y [9,15].

Immunization of mice with 71 kDa/30kDa-FIA induced protective immunity against a sublethal challenge of M.tuberculosis H_{37} Rv as demonstrated by increased survival and decreased viable counts in immunized group as compared to BCG vaccinated as well as controls. These findings are further substantiated by previous reports on the protective efficacy of mycobacterial protein antigens [7,8,16,17].

The long term acquired resistance observed in the study was slightly lower than short duration experiments (Fig.5). But it was still higher than BCG indicating the presence of stable state of memory on immunization with immunodominant mycobacterial proteins. The observed decline in protective immunity may be due to lack of persistence of antigen which is required to maintain the protective immunity as well as state of memory [18]. The encapsula

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tion of 30 kDa in liposomes induced significant cellular responses and protection afforded was equivalent to BCG (data not shown), thus suggesting that liposomes can effectively replace FIA. However, the immunoreactivity of 71 kDa protein decrease on encapsulation in liposome suggesting that the adjuvanticity of liposomes is affected by nature and type of antigen. Therefore, alternate adjuvant system such as PLG, DDA or SAF-1 should be evaluated. Further, the protective efficacy was found to be mediated by both CD4+ and CD8+T-cell cooperative effect suggesting the antigen presentation in association with MHC class I and II molecules [19,20] which was further substantiated by addition of Natural killer cells, dendriitic cells and macrophages present in the total spleenocytes. The immunoprotection imparted by both the protein antigens was found to be specific and no cross reactivity and cross protection (data not shown) was observed with unrelated intracellular pathogens (Salmonella, Aspergillus and Leishmania)

In brief, immunodominant proteins like 71 kDa cell wall associated protein and 30 kDa secretory protein having protective efficacy better than BCG vaccine are suitable candidates for subunit vaccine against tuberculosis. The evaluation of their combined effect in biodegradable carrier vehicles may be a step ahead in vaccine development.

Table - 1

Protection induced (on survival basis) in mice immunized with 71 kDa-FIA/30 kDa-FIA and challenged 8 weeks p.im. with LD_{50} of M.tuberculosis $H_{37}Rv$.

Group immunized with	Protective effect = Number of survivors/ Number of animals tests			
	7	14	21	30
71-kDa-FIA (60ug/mouse)	19/19(100)	19/19(100)	19/19(100)	17/19(86.5)
30-kDa-FIA (75ug/mouse)	15/15(100)	15/15(100)	15/15(100)	13/15(86.6)
BCG vaccinated	20/20(100)	20/20(100)	19/20(95)	14/20(70)
Control (Saline-FIA)	20/20(100)	20/20(100)	15/20(75)	10/20(50)

Figures in parentheses represent percent survival.

Table - 2

Viable bacilli counts from lung, liver and spleen of 71 kDa-FIA/30 kDa/ FIA immunized mice as compared to BCG vaccinated and control mice challenged at 8 week p.im. The viable bacilli enumeration was done after 30 days of challenge with LD_{50} of M.tuberculosis $H_{37}Rv$.

Group	Viable counts in		
	Lung	Liver	Spleen
71kDa-FIA immunized	$2.59\pm0.15x10^{6***!!!}$	$1.21\pm0.04x10^{5***!!!}$	$2.00+0.43x10^{5NS}$
30-kDa-FIA immunized	$2.8+0.4x10^{5***!!!}$	$1.8+0.8x10^{5**!!}$	$7.2+1.11X10^{5NS}$
BCG vaccinated	$3.97+0.43x10^{7***}$	$3.22+0.76x10^{6***}$	$1.84+0.65x10^{5NS}$
Normal saline-	$3.48\pm0.92x10^{8}$	$3.01\pm0.67x10^{7}$	$2.86\pm0.52x10^{5}$

Results are presented as mean of 4-5 animals and each group was repeated thrice.

***p<0.001, **p<0.01, *NS p < 0.05 Non significant decrease in test groups as compared to controls.

!!! p<0.001 decrease in 71 kDA-FIA/30 kDa-FIA immunized group as compared to BCG vaccinated groups

LD_{50} median lethal dose.

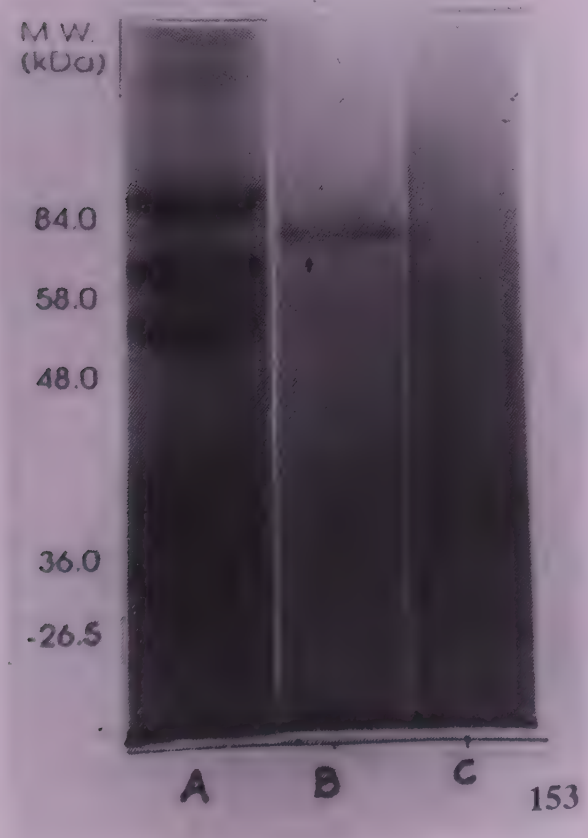


Fig.2 *SDS-PAGE of purified 71 kDa cell wall associated and 30 kDa secretory protein as compared to molecular weight markers. Triose isomerase (26.5 kDa), LDH-36kDa,Fumarase (48kDa), pyruvate kinase (84kDa) obtained from Sigma served a molecular weight marker. Lane A, molecular weight markers, Lane B-71 kDa and Lane C-30kDa.*

Fig 1 (a)

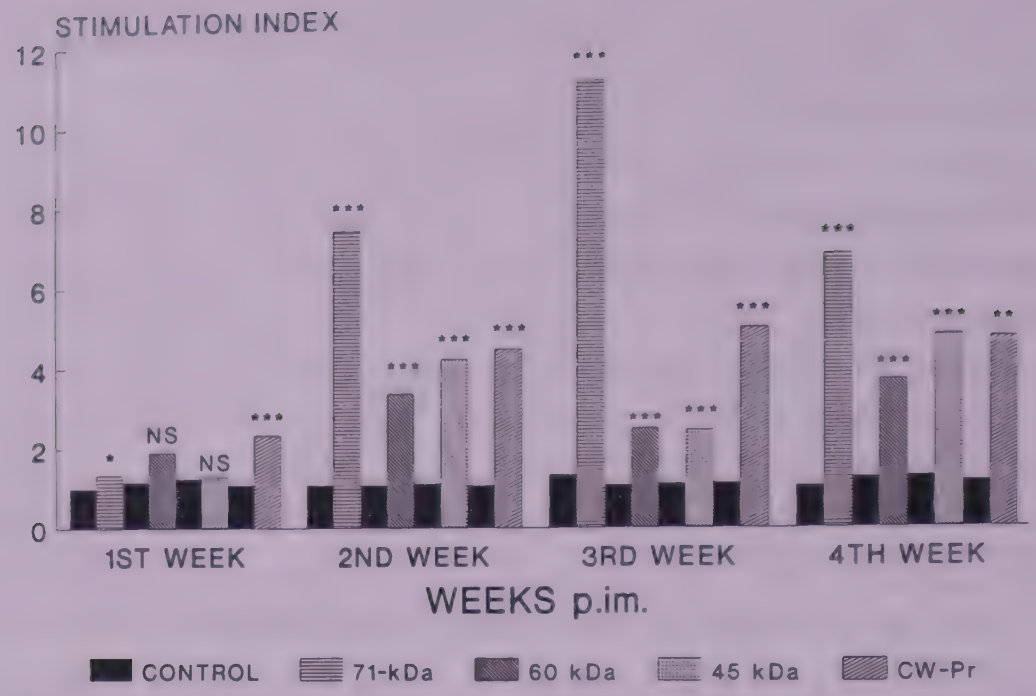


Fig 1 (b)



Fig. 1 *Comparative study of proliferative responses elicited in animals immunized with (a) total CW-Pr-F1A and (b) total culture filtrate protein F1A in response to isolated individual antigens. SI (stimulation index) is mean [^{3}H] incorporation in lymphocytes incubated with antigen/mean [^{3}H] incorporation in lymphocytes incubated without antigen. All values are mean of 4-5 animals.*

***p<0.01; ***p<0.01 with respect to control.*

Fig 3 (a)

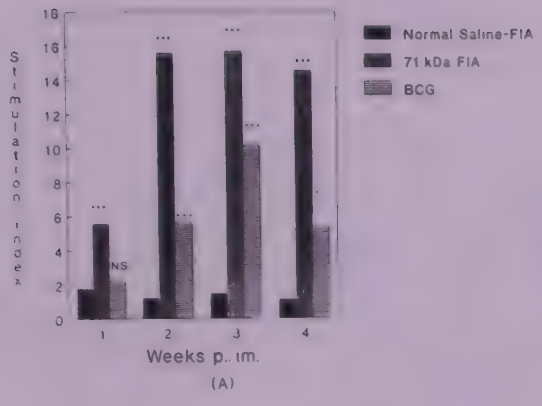


Fig 3 (b)

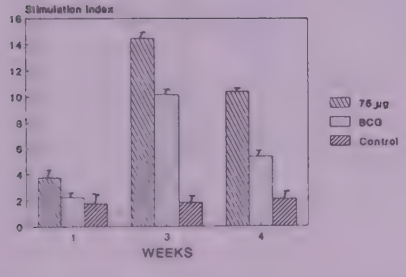
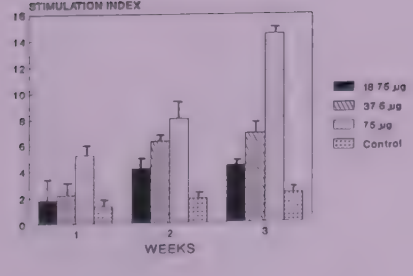


Fig 3 (c)



CONTROL

Fig.3 ***Comparison of T-cell proliferative response (a) to 71 kDa protein in mice immunized with 71 kDa-FIA (60 ug/mouse) (b) to 30 kDa in mice immunized with 30 kDa-FIA (75ug/mouse) in comparisin to BCG (c) T-cell proliferative responses obtained in mice immunized with different concentrations of 30 kDa antigen.***

Fig 4 (a)

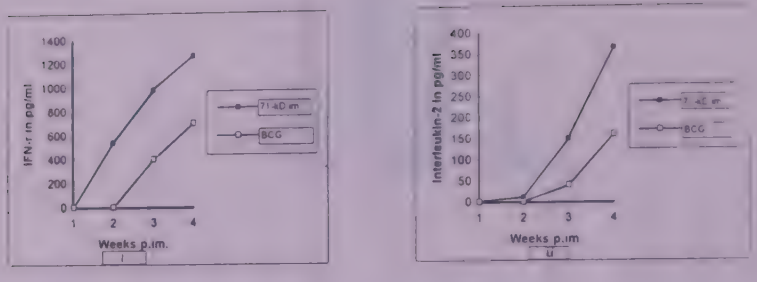


Fig 4(b)

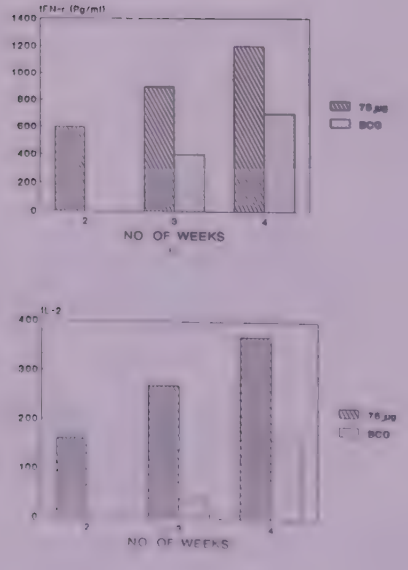


Fig. 4 *The levels of IFN-γ and IL-2 in culture supernatants of (a) 71 kDa and (b) 30 kDa sensitized lymphocytes obtained from mice immunized with 71 kDa/30kDa/BCG.*

Fig 5

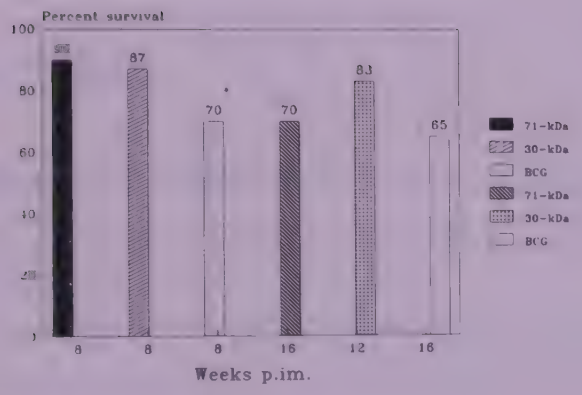


Fig. 5 *Comparison of percent protection afforded by immunization with 71 kDa FIA at 8 and 16 weeks p.im and 30 kDa-FIA at 8 and 12 weeks p.im and BCG against challenge with LD 50 of M.tuberculosis H37Rv.*

CONTROL

References:

1 Collin, F M Immunology of mycobacterial infections: In: Escobar, MR utz JP eds. Plenum press, New York, 1988: 125.

2. Barnes, P.F., Mehra, V., Hirschfield, G.R., Fong, S., Abou Zeid, C. Rook, GAW, Hunter, S.W., Brennan, P.J. and Modlin r.L. J.Immunol. 1989, 143, 2656-2662.

3. Hirschfield, G.R., McNeil, M and Brennan, P.J.J. Bacteriol. 1990. 172: 1005-1013

4. Abou Zeid, C., Smith, I., Grange, J.M., Ratiliff, T.L., Steele, J. and Rook, G.A.W.J.Gen.Microbiol, 1988, 134, 531-538.

5. Andersen, P., Askgaard, D., Ljungquist, L. Bentzon, M.W. and Heron, I., Infect. Immun. 1991, 59, 1558-1563.

6. Halvir, D.V., Wallis, R.S., Boom, W.H., Daniel, T.N., Chervenak, K. and Elner, J.J., Infect. Immun. 1991,59, 665-670.

7. Pal, P.G. and Horwitz, M.A. Infect. Immun. 1992, 60, 4781-4792.

8. Andersen, P. and Heron, I.Infect. Immun. 1993, 61, 844.

9. Andersen, P.Infect. Immun. 1994, 62, 2536-2544.

10. Dhiman, N., Verma, I. and Khuller, G.K.J.Biosci. 1997, 22, 1-9.

11. Sinha, R.K., Verma, I. and Khuller, G.K. Vaccine, 1997. (In press).

12. Orme, I.M.J. Immunol. 1987, 138-293-298.

13. Orme, I.M. and Collins, F.M., Cell. Immunol. 1984, 113-120.

14. Matsuo, K., Yamagauhi, R., Yamazaki, A., Tasako, H. and Yamada, T.J. Bacteriol, 1988, 170, 3847-3854.

15. Hannen, J.B.A.G., de wall Malefijt, R., and Res, PC.M.et al. J.Exp.Med. 1991, 174, 583-593.

16. Chugh, I.B. and Khuller, G.K.Eur, Resp. J.1993, 6, 811-815.

17. Orme, I.M. Clin. Exp. Immunol. 1988, 71, 388-393.

18. Nagai, S., Wiker, H.G., Harboe, M. and Kinomoto, M. Infect. Immun. 1991, 59, 372-382.

19. Flynn, J.L., Goldsteim, M.M., Triebold, K.J., Koller, B. and Bloom, B.R. Proc. Natl. Acad. Sci. 1992, 89, 12013-12017.

20. Young, D.B., Kaufmann, S.H.E., Herrons, P.W.M. and Thole, J.E.R. Mol. Microbiol. 1992, 6, 133-145.

The Private Medical Sector And Tuberculosis Control In India

Mukund Uplekar

In spite of its indisputable importance in general and capacity to influence programme implementation in particular, the private medical sector appears to have been allowed to grow unchecked and yet been kept out of plans, policies and programmes related to purposeful development of health services in India. Reasons for this are unclear but the results are too well known.

This paper presents the findings of some of the first studies on the private sector in tuberculosis control in India, undertaken by the Foundation for Research in Community Health , in the rural and urban parts of Maharashtra. Two studies examined the management practices of private medical practitioners. One prospective study documented the treatment behaviour of TB patients under care of private medical practitioners and the third one evaluted two city-based TB projects undertaken by groups of private medical practitioners.

A benign private sector catering chiefly to the psychological needs of the people even at an immoderate cost but with out causing much damage to public health concerns could be disregarded, ignored, or tolerated. This however is not the case . It is becoming increasingly clearer that the private sector has anything but a worthwhile influence in improving health or controling diseases of Indian people. The onus of this however falls equally on both , the

former for being unresponsive to peoples expectations and driving them away to private sector and the latter for being insensitive to larger repercussions of their actions.

Identification, experimentation, and wider application of specific interventions to elicit meaningful contribution of the private sector for effective implementations of the national tuberculosis programme could not only further the objectives of the programme significantly but also help envision steps towards achieving and offering a more comprehensive public-private mix of health care service to the people. In designing such interventions, the larger interests of the people ought to take precedence over the narrow concerns of either the private or the public health personnel.

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Mr.J.S.Jungade

INTERNATIONAL CME ON TUBERCULOSIS

PROGRAMME

27th Sept. 1996 — **Day - I**

7.30 A.M. to 8.30 A.M. — Breakfast — *Venue: Community Hall*

8.00 A.M. to 9.00 A.M. — Registration of CME & Conference

Venue : Auditorium

9.00 A.M. to 9.45 A.M. — Inauguration

9.45 A.M. to 10.30 A.M. — **Keynote Address**
Chairpersons: **Dr.Sushila Nayar**
Dr.O.P.Gupta
Newer Drugs & Newer Strategies in the Treatment of Tuberculosis — **Dr.P.R.J.Gangadharam**

10.30 A.M. to 11.00 A.M. — Tea — *Venue: J.B.T.D.R.C.*

Venue: Anatomy Lecture Hall

Session I

11.00A.M. to 11.45 A.M — Chairpersons: **Dr.Mukund Uplekar**
Dr.B.S.Garg
Epidemiology of Tuberculosis in India — **Dr.A.K.Chakraborty**

11.45 A.M. to 12.30 P.M. — Chairpersons: **Dr. Pratibha Dalal**
Dr. D. K. Mendiratta
HIV and tuberculosis — **Dr.D.Sengupta**

12.30 P.M to 1.00 P.M. — Chairpersons: **Dr.Pushpa Jagtap**
Dr. P. Narang
Typing of Mycobacterium Tuberculosis with special Reference to biotyping — **Dr.(Mrs)S.Kothadia**

1.00 P.M. to 2.30 P.M. — Lunch — *Venue: Community Hall*

Venue: Anatomy Lecture Hall

Session II

2.30 P.M. to 3.15 P.M. — Chairpersons: **Dr.A.K.Chakraborty**
Dr.U.N.Jajoo
Why are we still using BCG? — **Dr.J.L.Stanford**

3.15 P.M. to 3.30 P.M. — Tea

27th Sept. 1996 **Day-1**

Session III

Chairpersons: **Dr.V.L.Jahagirdar**
Dr.S.P.Kalantri

3.30 P.M.	to	*Drug Resistance in*	**Dr.P.R.J.Gangadharam**
4.15 P.M.		*Tuberculosis*	
4.15 P.M.	to	*Prediction of Relapse in*	**Dr.(Mrs)M.Pathak**
4.45 P.M.		*Pulmonary Tuberculosis-*	
		An Epidemiological Model	
5.00 P.M.		Prayer	*Venue: Adhyayan Mandir*
7.00 P.M.		Light Entertainment programme	*Venue: Auditorium*
8.30 P.M.		Dinner	*Venue: Community Hall*

28th Sept. 1996 **Day-II**

7.30 A.M.	to	Breakfast	*Venue: Community Hall*
8.30 A.M.			

Venue: Anatomy Lecture Hall

8.30 A.M.	to	**Keynote Address**	
9.15 A.M.		Chairpersons: **Dr.P.R.J.Gangadharam**	
		Dr.A.P.Jain	
		Immunotherapy in Tuberculosis Myth	**Dr.J.L.Stanford**
		or reality	

Session I

Chairpersons: **Dr.V.N.Chaturvedi**
Dr.A. Mehendale

9.15 A.M.	to	*Short course Chemotherapy in*	**Dr.(Mrs)P.Chaturvedi**
9.45 A.M.		*Children*	
9.45 A.M.	to	*Problem of Tuberculosis and*	**Dr.A.K.Chakraborty**
10.15 A.M.		*NTP in India*	
10.15 A.M.	to	*Role of Private providers in Health*	**Dr.M.Uplekar**
10.45 A.M.		*Services for Tuberculosis Programme.*	
10.45 A.M.	to	Tea	
11.00 A.M.			
11.00A.M.	to	Exhibition on Tuberculosis	Inaguration by
12.15 PM			**Mrs.Cynthia Stanford**

Session II

12.15 P.M.	to	Chairperson : **Dr.Sushila Nayar**	
		Tuberculosis in Bombay	**Dr.Pratibha Dalal**
1.00 P.M.	to	Lunch	
2.00 P.M.			

Session III

Chairpersons: **Dr.D.V.Kaundaniya**
Dr.V.C.Ohri

2.00 P.M.	to	*Recent Advances in Diagnosis*	**Dr.P.R.J.Gangadharam**
2.45 P.M.		*of Tuberculosis*	
2.45P.M.	to	*Characterisation of Mycobacterium*	**Dr.T.Kamala**
3.15 P.M.		*Avium Complex (MAC) Isolates Obtained from the Environment of the South Indian BCG Trial Area.*	
3.15 P.M.	to	Tea	
3.30 P.M.			

Session IV

Venue: Anatomy Lecture Hall

Chairpersons : **Dr.J.L.Stanford**
Dr.K.V.Desikan

3.30 P.M.	to	*Mycobacterial Protein Antigens*	**Dr.G.K.Khullar**
4.00P.M.		*as Immunoprophylactic Agents against Experimental Tuberculosis*	

Session V

Venue : Radiology Department

3.30 P.M.	to	*Radiology in Tuberculosis*	**Dr.P.N.Sharma**
4.30 P.M.			
4.30 P.M.		Departure for Bor Dharan	*From Registration*
8.00 P.M.		Dinner	*Venue :Bor Dharan Rest House*
9.30 P.M.		Return to Sevagram	

A Report on International CME on Tuberculosis : Need for Newer Strategies in Control of Tuberculosis

An International Continuation Medical Education Programme (CME) on Tuberculosis was organised by the Department of Microbiology, M.G.I.M.S., Sevagram, Wardha on 27th & 28th Sept., 1996. The programme was well attended by 142 delegates, and included Microbiologists, Clinicians and Practicising physicians from all over Maharashtra and other states of India. Sixteen eminent scientists from India and abroad joined as guest faculty. The main theme of the CME was to cover some of the recent advances in the epidemiology, diagnosis, treatment and control of tuberculosis.

The function was inaugurated by Dr. P.R.J.Gangadharam, Professor of Medicine and Microbiology & Pathology, Director of Mycobacteriology Research at the University of Illinois, Chicago, USA, also a recipient of prestigious Robert Koch Centennial and Burrows Wellcome awards.

Dr. J.L.Stanford, Head of Bacteriology University College, London Medical School, U.K. and Dr. A.K. Chakraborty (Retd.)Additional Director, National Institute of Tuberculosis, Bangalore were the guests of Honour.

Dr.Sushila Nayar, Director, MGIMS, presided over the function. The Dean, O.P.Gupta welcomed the guests and Dr.Mrs.P.Narang, Professor & Head, Deptt. of Microbiology and Organising Secretary of the CME while introducing the objectives of the CME emphasised its need in the present day situation when HIV and tuberculosis existed in a deadly combination and when every

second some one in the world was getting infected with the disease.

Dr. Sushila Nayar, in her address stressed the need for periodic review on the problems in the management of tuberculosis and the need for such CME programmes for providing an Update.

Dr.P.R.J. Gangadharam in his inaugural keynote address dealt in detail about the need for newer drugs in the treatment of tuberculosis and highlighted the use of newer strategies in drug delivery methods like use of Liposomes and biodegradable polymers as depots.

A Souvenir was released by Dr. A.K. Chakraborty. The artistic portrait of Gandhiji on the cover page truly reflected Gandhiji's interest in scientific knowledge and persuit for its truthful application. Dr.Deepak Mendiratta, Joint Secretary proposed a vote of thanks.

Dr.Stanford delivered talk on the role of immunotherapy in tuberculosis. He narrated the success story of various trials carried out by him and his colleagues in different parts of the world. He also discussed how an antigen prepared from an innocuous bacterium could boost the body resistance and could provide support to anti tuberculosis treatment.

Dr. Mrs. P. Chaturvedi, Professor & Head, Deptt. of Paediatrics, MGIMS, Sevagram spoke on short term treatment of tuberculosis in children.

Dr. A.K.Chakraborty covered the National Tuberculosis Programme and the epidemiology of tuberculosis in the light of prevalence, difficulties encountered in case detection and diagnosis and compared the Indian Scenario with that of WHO expectations. He also spelt out the new strategies under the revised National with Tuberculosis Programme and need for supervised treatment in order to achieve effective results and control of tuberculosis. The role of private practitioner in the control of tuberculosis was stressed upon by Dr. Uplekar from Foundation for Research in Community Health, Bombay. Dr. Mrinalini Pathak , Professor of Community Medicine, J.N.M.C., Sawangi informed about an epidemiological model devised by her to predict defaulters in the treatment of pulmonary tuberculosis . Dr.Sengupta, from National AIDS Control Organisation gave very informative talk on

the global problem of HIV and stressed on the need for vigilance and follow up studies in order to contain the impending global epidemic of deadly dual infection.

The session on recent advances in Laboratory diagnosis of tuberculosis was covered by Dr.Gangadharam. He spoke at length on recently introduced rapid methods for diagnosis of tuberculosis. Dr.B.C.Harinath, MGIMS, Sevagram highlighted some of the methods used for immunodiagnosis and Dr.G.K.Khuller from PGI, Chandigarh informed about some antigens of Mycobacteria as potential candidates for vaccines in tuberculosis. Dr.Kothadia from Solapur emphasised an epidemiological typing of M.tuberculosis strains with particular reference to simple biotyping method; while Dr.T.Kamala from Tuberculosis Research Centre, Madras talked about the isolates from Madras. A separate session on Radiology of Tuberculosis was organised and conducted by Dr.P.N.Sharma, Professor and Head Deptt. of Radiology, MGIMS, Sevagram.

The CME concluded on the vote that concerted efforts in all directions were needed to nip the ugly head of tuberculosis in the bud. While efforts were necessary in bringing out potent vaccines & devising rapid and effective methods for early detection of cases, the most imporant thrust area is still proper supervised treatment. The question whether boosting body resistance by vaccines or depot drugs delivery system would be the answer to better compliance and cure, the furure only will tell. But at present the most important step would be to make the present potent bactericidal drugs available for treatment and to ensure their total intake. This single strategy, the conference concluded, would go a long way in promoting rapid sputum negativity, decline in communicability and put brakes on development of multidrug resistance in strains of Mycobacterium tuberculosis.

List of Participants

	Name	City
1.	Dr. Agrawal G.N.	Nagpur
2.	Dr. Angadi Subhash A.	Mumbai
3.	Dr. Babu R.C.	Calicut
4.	Ms.Balgam Shilpa S.	Solapur
5.	Dr.Ballal Rajesh M.	Nagpur
6.	Dr.Banait Kumar Nand	Sevagram
7.	Dr.Banait Pramodchandra	Nagpur
8.	Mr.Banerjee Kaushik H.	Solapur
9.	Mr.P.J.Barve	Mumbai
10.	Dr.(Mrs.) Basak S.S.	Wardha
11.	Dr.Bedarkar Kalpana	Nagpur
12.	Dr.Bhalerao Deepika	Miraj
13.	Ms.Bhosale Shailaja	Solapur
14.	Dr.Bramhne H.G.	Raipur
15.	Dr.Chakraborty A.K.	Bangalore
16.	Mr.Chalwadi Prashant	Solapur
17.	Mrs.Chattopadhyay Dharmaja	Mumbai
18.	Dr.Mrs.Chaturvedi P.	Sevagram
19.	Dr.Chowdhary Abhay S.	Mumbai
20.	Dr.Dahat Shilpa	Mumbai
21.	Dr.Dalal P.J.	Mumbai
22.	Miss. Dalal Sunali B.	Mumbai
23.	Ms. Das Sharmishtha	Mumbai
24.	Dr. Deotale Vijaysree	Sevagram
25.	Dr. Deshmukh A. B.	Aurangabad
26.	Dr. Deshmukh Pradeep	Sevagram
27.	Dr. Deshpande Mukta	Wardha
28.	Dr. Dey Sangeeta	Sevagram
29.	DP Medical Diagnositcs PVt.Ltd.	Mumbai
30.	Miss. Dwiwedi Garima	Pune
31.	Dr. Miss Fule C.P.	Yavatmal
32.	Dr. Gaikwad P.Y.	Nagpur
33.	Dr. Gallot Sunil	Sevagram
34.	Dr. Gangadharam P.R.J.	USA
35.	Dr. Garg Naresh	Sevagram

	Name	City
36.	Ms. Ghodke Urmila P.	Pune
37.	Dr. Govinda P.	Calicut
38.	Dr. Harikrishnan R.	Sevagram
39.	Dr. Harinath B.C.	Sevagram
40.	Dr.Ingole K.V.	Yavatmal
41.	Dr. Iyer Ranganathan N.	Mumbai
42.	Dr. Mrs. Jagtap P.M.	Nagpur
43.	Dr. Jahagirdar Vilas	Solapur
44.	Dr. Jaitty N.K.	Nagpur
45.	Dr. Jalgaonkar S.V.	Yavatmal
46.	Dr. Joshi Sandhya	Solapur
47.	Ms. Joshi Suvarna Aniruddha	Pune
48.	Dr. Kagal Anju	Pune
49.	Dr.Kamala T.	Bangalore
50.	Mr.Kamble Ravindra	Solapur
51.	Dr.Kamble R.W.	Nagpur
52.	Miss Kambli Anjali Manohar	Mumbai
53.	Dr. Mrs. Kamlakar Usha	Nagpur
54.	Dr. Kanti A.	Beed
55.	Lt.Col.(Mrs.) Kapila K.	Pune
56.	Dr. Karadesai S.G.	Belgaum
57.	Dr. Kaundinya D.V.	Mumbai
58.	Dr. Khadse R.K.	Nagpur
59.	Dr. Khuller G.K.	Chandigarh
60.	Dr. Kothadia Sarita	Solapur
61.	Dr. Kulkarni Sanjay	Solapur
62.	Ms.Kulkarni Anita	Solapur
63.	Mr.Kulkarni Parag A.	Pune
64.	Dr. Kulkarni Sadanand Sudhakerrao	Pune
65.	Mr. Kulkarni Milind	Solapur.
66.	Dr. Kulkarni Vishwas Vasudev	Sangli
67.	Dr. Kumar Niraj	Gwalior
68.	Ms.Lele Swati	Solapur
69.	Ms.Loraine Sequeria	Mumbai
70.	Dr. Mangalkar Suvarnalata	Nanded.

	Name	City
71.	Dr. Mani Rita	Miraj
72.	Dr. Mendiratta D.K.	Sevagram
73.	Dr. Meshram S.P.	Nagpur
74.	Mr. Mishra S.N.	New Delhi
75.	Dr. Mistry Nerges F.	Mumbai
76.	Dr. Mokashi Nitin D.	Pune
77.	Dr. Mundhadha Sheela	Nagpur
78.	Dr. Munje R.P.	Nagpur
79.	Dr.Mrs.Nagdeo Neena V.	Nagpur
80.	Dr.Nagdeve Varsha	Nagpur
81.	Dr. Narang Rahul	Sevagram
82.	Dr. Narang Pratibha	Sevagram
83.	Dr. Nayyar Aruna	Sevagram
84.	Dr. Nikam Vrishali Vijay	Pune
85.	Dr. Palshikar Manasee Girish	Pune
86.	Dr. Parab Jairam	Panaji
87.	Dr. Parikh Trupti	Baroda
88.	Dr. Patankar Jyoti V.	Nagpur
89.	Dr. (Mrs.) Pathak Mrinalini	Wardha
90.	Dr. Pati S.S.	Sevagram
91.	Dr. Patil Vyankatesh Y.	Malegaon
92.	Dr. Pillai A.P.	Nagpur
93.	Ms. Pilley Pratibha	Solapur
94.	Dr. Pramanik J.	Sevagram
95.	Dr. Pundker S.R.	Wardha
96.	Dr. Quazi M.S.	Nagpur
97.	Dr. (Mrs.) Rao Alka	Jaipur
98.	Mrs. Rathod V.S.	Nanded
99.	Dr. Mrs. Raut Sharmila	Nagpur
100.	Dr. T.Rupamkumar	Nagpur
101.	Dr. Sahasrabudha Sampada H.	Pune
102.	Dr. Sami M A	Sevagram
103.	Dr. Saraf A.A.	Nagpur
104.	Dr. Sarnaik Ravindra M.	Nagpur
105.	Dr. (Mrs.) Sati Pathak	Jaipur
106.	Dr. Sengupta Shubhra	Mumbai

	Name	City
107.	Dr. Sengupta D.	New Delhi
108.	Dr. Set Reena	Mumbai
109.	Dr. Shah Prashant H.	Baroda
110.	Mr. Shahram Masoud	Pune
111.	Dr. Shahriar Roushani	Miraj
112.	Mr. Sharma	New Delhi
113.	Ms. Sharma Hema	Solapur
114.	Dr. Mrs.Sheikh Nasira	Solapur
115.	Dr. Shetty Vidya H.	Mumbai
116.	Dr. Shinkre Nandita B.	Mumbai
117.	Dr. Shivananda B.P.	Belgaum
118.	Dr. Shrikhande Sunanda N.	Nagpur
119.	Dr. Sijwali Mahinder	Sevagram
120.	Dr. Singh Sharatchander L.	Sevagram
121.	Dr. Singh U.B.	Purnia
122.	Dr. Srikant	Bangalore
123.	Dr. Stanford J.L.	London
124.	Mrs. Stanford Cynthia	London
125.	Mr. Singh Sukhdev	New Delh
126.	Dr. Sukhdeo Kuldeep	Sevagram
127.	Dr. Tangri Rajiv	Sevagram
128.	Dr. Tankhiwale N.S.	Nagpu
129.	Dr. Mrs.Tankhiwale S.S.	Nagpu
130.	Dr. Thakur Archana	New Delh
131.	Ms. Tonse Sarla	Solapu
132.	Dr. Udgaonkar Usha	Mira
133.	Dr. Ujgare Mahadeo Tukaram	Aurangaba
134.	Dr. Upadhyay Sanjeev	Sevagra
135.	Dr. (Mrs.) Upadhyaya Sunita	Lucknow
136.	Dr. Uplekar Mukund	Mumba
137.	Ms. Uppe Archana	Mumba
138.	Dr. Vaidya Jayshree	Sevagra
139.	Dr. Warade V.B.	Nagpu
140.	Dr. Wasekar Varsha	Sevagra
141.	Dr. Yadav Balraj	Sevagra
142.	Dr. Zodpey Sanjay P.	Nagpu



Participants